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Inherited and rearing components of aggressive dominance and autonomic reactivity in the rat

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Abstract. On the basis of their scores in the open field test apparatus and 'limited access' competition, rats were divided into high and low aggressive and high and low reactive lines. Intrastrain breeding experiments were conducted across 3 generations to determine the heritability of aggressive behaviour and reactivity. In each generation, the animals of each strain were reared under 'normal', 'crowded' and 'food restricted' conditions to determine the extent of the influence of rearing environment on these behaviours. The genetic and environmental contributions to aggression and reactivity were further investigated through reciprocal cross matings and foster rearing. In most of the cases, environmental factors showed significant but varied and unpredictable influences, whereas the genetic factors were more consistent in their effects.

Keywords. Aggressive behaviour; autonomic reactivity; inheritance; environmental influences.

1. Introduction

Aggression is a catch-all term and its expression may include components which could be physiological, emotional or cognitive. Behaviour geneticists have shown a considerable interest in the inheritance of various aspects of aggression (Taylor 1980; Broida and Svare 1982; Moss *et al* 1982) and 'high' and 'low' aggressive lines have been established in laboratory animals (Hyde and Sawyer 1980). Further studies involving diallel cross matings have shown the stability of inheritance of aggression on the one hand (Hahn and Huber 1982) and sex-related differential inheritance patterns on the other (Hood and Cairns 1988). On the other hand, experience and learning during development were found to significantly influence aggressive behaviour (Wuensch and Cooper 1981). Two environmental factors, population density (Calhoun 1962; Leyhausen 1965; Aspey 1977) and food availability and distribution (Boccia *et al* 1988) are known to directly affect aggressive behaviour.

Autonomic reactivity or emotionality is 'a complex of factors ... a group of organic, experiential and expressive reactions ... general upset or excited condition of the animal' (Hall 1934). A number of studies (DeFries *et al* 1978; Webster *et al* 1979; Marangos *et al* 1987) have shown a strong genetic component in autonomic reactivity which may suggest the possibility of establishing 'high' and 'low' lines through selective breeding. However, developmental conditions such as rearing in small litters (Akuta 1979), crowding (Levitt and Bennet 1975) and handling during infancy (Ader 1968) etc. also influence reactivity to a considerable extent.

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A relationship has been shown to exist between aggression and emotionality (Yumatov *et al* 1988). In most of the earlier studies, both genetic and environmental factors have been shown to affect aggressive behaviour and reactivity. The present study was designed to assess the relative contribution of genetic and environmental factors on the expression of aggressive behaviour and autonomic reactivity. The terms aggression or aggressive dominance refer to the expression of overt dominance in competition situations involving limited access to a resource. Although 'limited access' technique of measurement of aggression was criticized by Syme *et al* (1974), Pillai and Singh (1984) re-established 'limited access' to be a reliable measure. The technique has also been successfully employed by other investigators (Benton *et al* 1980; Boccia *et al* 1988).

2. Methods

2.1 *Subjects, experimental design and procedure*

A total of 482 albino rats of the Wistar stock served as subjects.

2.2 *Experiment 1: Parental generation*

Rats (126) were tested for autonomic reactivity and aggressive dominance in competitive situations. Based on their scores, the animals were divided into 4 groups to establish 4 strains, viz. high aggressive-high reactive (HA-HR), high aggressive-low reactive (HA-LR), low aggressive-high reactive (LA-HR) and low aggressive-low reactive (LA-LR). Males and females were, then, subjected to intrastrain breeding.

2.3 *Experiment 2: First generation*

The offspring from each strain were distributed into 3 rearing conditions:

Condition A: Normal rearing—one individual per small cage ($25 \times 10 \times 16$ cm).
Condition B: Space restricted (crowded)—8 individuals of like sex per large cage ($36 \times 30 \times 22$ cm).
Condition C: Food restricted—3 rats of like sex per large cage, provided, on average, 2 g per rat lesser food per day than the amount normally required at any age. The food was provided through an access available to only one rat at a time.

This way, 4 strains, 3 rearing conditions in each strain and sex, formed a $4 \times 3 \times 2$ factorial design (for reactivity). Sex, however, as an independent variable was not considered in aggressivity since males and females were not pitted against each other in competitive situations. At the age of about 80 days, the animals were first tested for autonomic reactivity and immediately after for aggressive dominance. Tests for aggressive dominance were conducted as follows.

2.3a *Intrastrain-intercondition tests*: Animals of the same strain but from different rearing conditions competed with each other.

2.3b *Intracondition-interstrain tests*: Animals from like rearing conditions but belonging to different strains competed with each other.

After the testing was over, the first generation animals were subjected to strain breeding in order to obtain the second generation stock.

2.4 *Experiment 3*: *Second generation*

The second generation experiment was a repetition of the first generation experiment.

2.5 *Experiment 4*: *Reciprocal crosses*

HA-HR and LA-LR animals of experiment 3 were subjected to reciprocal cross mating in the following manner: (i) HA-HR female × HA-HR male, (ii) LA-LR female × LA-LR male, (iii) HA-HR female × LA-LR male and (iv) LA-LR female × HA-HR male. The offspring, thus consisted of 2 inbred and 2 hybrid groups. The animals in 4 groups were raised in identical manner as 2 animals of like sex per cage.

At the age of 85 days (± 2 days), the animals were tested for autonomic reactivity. For the aggression tests, the animals from 4 different groups competed with each other in pairs and groups of 4, for the 3 competitive tests. Statistical analyses were carried out not only to assess the relative contribution of inbreeding and cross breeding, but also of male and female parentage in cross breeding to the total variance in behaviour.

2.6 *Experiment 5*: *Foster rearing*

HA-HR and LA-LR animals of experiment 3 were subjected to inbreeding. For the rearing purpose, the obtained offspring were distributed into 4 groups in the following manner: (i) offspring of HA-HR parents—reared by HA-HR mothers, (ii) offspring of LA-LR parents—reared by LA-LR mothers, (iii) offspring of HA-HR parents—reared by LA-LR mothers and (iv) offspring of LA-LR parents—reared by HA-HR mothers. After weaning, the animals were formed into two groups of 'mother-reared' and 2 groups of 'foster reared' subjects.

2.7 *Methods for assessment of autonomic reactivity and aggressive dominance*

2.7a *Autonomic reactivity*: The open field test apparatus was used to measure autonomic reactivity. The animal was exposed to light and sound stressors for 2 min each day for 5 days. Autonomic reactivity was determined by taking the mean defecation score obtained through the 5-day test.

2.7b *Aggressive dominance*: Measures of aggressive dominance were obtained in tests involving competition for food or water. Three tests, each after 48 h of deprivation, were conducted as follows:

(i) Pair-wise competition for food (15 min).

(ii) Group competition (4 animals at a time) for food (15 min).
(iii) Group competition for water (15 min).

Two-day regular feeding schedule was maintained between the tests.

The competition was for the source (food or water) which was accessible to only one animal at a time. Although each rat was involved in all the 3 tests, no animal was made to compete with another animal more than once in order to avoid the influence of any possible dominance hierarchy established during the previous competition.

During the experiment on parental generation, the variables recorded were body-weight before and after deprivation, body weight after the competition, time spent at the source during the contest and successful and unsuccessful attempts at displacing the competing rats from the source. These data were subjected to repeated intercorrelations, and it was established that the 'weight gain' and 'time spent' at the source during the contest were the most reliable measures of aggressive dominance. The details of these calculations are reported elsewhere (Pillai and Singh 1984). A single aggressivity score for an animal was calculated as follows:

(i) $c = \frac{\text{Total time spent at the source by all animals}}{\text{Total gain in body weight of all animals}}$.

(ii) Aggressive dominance score $=$ gain in body weight $+ \frac{\text{Time spent at the source}}{c}$

for a single animal.

c is a constant which indicates amount of food ingested per unit time by any animal irrespective of level of aggressivity.

2.8 *Apparatus*

2.8a *Open field test apparatus*: The apparatus consisted of a circular arena, 82 cm in diameter and 32 cm in height, made of hard cardboard. The white floor was marked into segments of equal area in a series of 3 concentric circles. The arena was placed in a 60 cm high, semi-sound insulated box. A 0·7 cm thick transparent glass covered the box. The stress stimuli were 8 bulbs of 100 watts each and high pitch sound generated on an audio-oscillator and fed to a loud speaker via an amplifier. A time switch controlled the duration of light and sound.

2.8b *Limited access test cage*: The test cage had dimensions of 36 × 30 × 22 cm and was made of thick wire mesh, which facilitated direct observations from all sides and top. The access which was situated at the back of the cage measured 4 × 4 cm and allowed only a single rat to the resource at a time. The access was kept shut with a sliding plate which was removed only at the onset of the experiment.

3. Results

3.1 *Experiment 2*: *First generation*

3.1a *Autonomic reactivity*: The obtained F values were significant for strain ($F =$

6·07; $P<0·01$), reactivity ($F=14·74$; $P<0·01$), rearing ($F=3·73$; $P<0·05$), sex ($F=9·06$; $P<0·01$) and strain and sex interaction ($F=3·34$; $P<0·05$). HA-HR and LA-HR strains (means 2·49 and 2·61 respectively) scored significantly higher on reactivity than LA-LR strain (mean 1·38). Irrespective of the aggressivity background, the descendents of HR parents scored significantly higher than the descendents of LR parents (means 2·55 and 1·67 respectively). With regard to the effect of rearing conditions, the crowded and the food restricted rats (mean 2·21 and 2·43 respectively) were more reactive than the rats reared under normal condition (mean 1·69). A significant strain × sex interaction revealed that whereas among males HA-HR strain scored highest on reactivity, the same happened in LA-HR strain among the females.

3.1b *Aggressive dominance*: *Intrastrain-intercondition tests*: The analysis indicated a highly significant effect of rearing conditions ($F=116·39$; $P<0·01$) and rearing × sex interaction ($F=10·78$; $P<0·01$) on aggressive dominance scores. The scores on aggressive dominance by crowded rats (mean 16·38) and food restricted rats (mean 15·47) were significantly higher than those of normally reared rats (mean 9·21). A significant rearing × sex interaction revealed that the effect was stronger on males than on females (mean scores: females; 8·64 – normal, 13·90 – crowded, 12·72 – food restricted: males; 9·79 – normal, 18·87 – crowded, 18·22 – food restricted).

3.1c *Intracondition-interstrain tests*: The analysis indicated that 4 major strains as well as 'aggressivity' as a strain differed significantly amongst each other ($F=3·87$; $P<0·05$ and $F=10·27$; $P<0·01$ respectively). The mean score (15·35) of 'HA' (descendents of HA parents) animals was significantly higher than the mean score of 13·71 of 'LA' (descendants of LA parents) animals. The '*t*' tests indicated that HA-HR and HA-LR strains had higher aggressivity scores (means 15·61 and 15·10 respectively) than LA-HR (mean 13·34) and LA-LR (mean 14·05) strains.

3.2 *Experiment 3*: *Second generation*

The second generation experiment was a repetition of the first generation experiment to re-confirm the findings.

3.2a *Autonomic reactivity*: As far as the major strains and 'reactivity' as a strain were concerned, the results in the second generation experiment were the same as in first generation. The mean reactivity score of HR animals (3·07) was significantly higher than the mean score of LR animals (mean 1·86), indicating inheritance of autonomic reactivity. Rearing conditions, however, failed to show any significant effect on reactivity.

3.2b *Aggressive dominance*: The analysis of variance for the data obtained through intrastrain-intercondition and interstrain-intracondition tests revealed the findings to be identical to those of experiment 2-first generation experiment, of course with different individual and mean values.

3.3 *Experiment 4*: *Reciprocal crosses*

3.3a *Autonomic reactivity*: The analysis of variance computed for autonomic reactivity revealed no differences among the mean scores of the 4 groups ($F = 0{\cdot}664$).

3.3b *Aggressive dominance*: The analysis revealed that the mean difference among the strains for aggressive dominance was statistically significant at 0·05 level ($F = 3{\cdot}92$). A further analysis indicated that the offspring of both HA parents scored higher on aggressive dominance (mean 14·24) than the other 3 strains (HA-HR × LA-LR = 10·04; LA-LR × HA-HR = 9·61; LA-LR × LA-LR = 9·44).

3.4 *Experiment 5: Foster rearing*

3.4a *Autonomic reactivity*: The analysis revealed significant differences between the HR and LR strains (means 3·09 and 1·72 respectively), between mother and foster rearing (means 2·01 and 2·80 respectively) and among the 4 groups ($F = 6{\cdot}33$; $P < 0{\cdot}01$). The HR strain animals reared by their biological mothers obtained higher scores (mean 2·88) than the LR strain animals reared by their own mothers (mean 1·13). The foster reared HR strain (mean 3·33) scored higher than the mother reared LR strain, and the LR strain foster reared by HR females (mean 2·30) obtained higher scores than the LR mother reared strain. Foster rearing enhanced the reactivity scores in both the strains, but the effect was more striking in the case of LR animals reared by HR females than in the HR animals foster reared by LR females.

3.4b *Aggressive dominance*: The analysis indicated the F values to be significant for strains ($F = 4{\cdot}32$; $P < 0{\cdot}05$), groups ($F = 8{\cdot}03$; $P < 0{\cdot}01$), strain × rearing interaction ($F = 16{\cdot}90$; $P < 0{\cdot}01$) and strain × rearing × sex interaction ($F = 5{\cdot}57$; $P < 0{\cdot}05$). The HA strain animals reared by their own mothers (group I, mean 13·13) scored significantly higher than LA animals reared by their own mothers (group III, mean 7·61). HA animals reared by LA females (group II, mean 10·98) also scored higher than group III. LA animals reared by HA females (group IV, mean 12·79) scored significantly higher than group III animals of the same strain. The significance of the strain × rearing interaction can be seen by comparing the means of group I with group II and of group III with group IV. Whereas in the HA strain, foster rearing by LA females reduced the aggressivity, the difference between the 2 groups was not significant. On the other hand in the LA strain, the foster rearing by HA females significantly increased the aggressivity. The 3-factor level significant interaction for strain × rearing × sex was evident from the means of various groups. The effect of foster rearing was absent in HA strain in both sexes. Furthermore, in the LA strain the foster rearing significantly increased aggressivity only in the case of males (means 6·09 and 14·52, 't' = 3·60), whereas the effect in the case of females was statistically non significant.

4. Discussion

4.1 *Autonomic reactivity*

The animals in the parental generation were divided into HR and LR groups on the basis of their scores in the open field test apparatus. In both first and second generation experiments, the offspring born to HR parents scored significantly higher on reactivity than the offspring born to LR parents. As far as inheritance of reactivity was concerned, the genetic determination showed up clearly, irrespective of the aggressivity of the animals in the 4 strains. Emotionality, being reactivity of autonomic nervous system, though modifiable to some extent by environmental factors, is more or less a physiological trait. The genetic determination of such behaviour traits that have strong physiological correlation is very much expected.

In the first generation experiment, crowding and food restriction significantly enhanced the reactivity when compared with normal rearing, irrespective of the genetic background. This finding also corresponds to the earlier studies mentioned in the 'introduction' regarding the nature of environmental factors influencing reactivity. The added feature of the present study was a repetition of the experiment using the same design. Surprisingly, in the second generation, the same environmental conditions failed to affect the reactivity of the animal. The results indicate that environmental influences are varied and unpredictable, and unlike inheritance of such traits, generalizations cannot be made even for as severe conditions as the ones employed in the present study.

The outcome in the reciprocal cross experiment was extremely interesting. Although the inbred HR and LR groups had significantly higher and lower scores respectively than the two cross bred strains, the differences were not statistically significant. These results add two more observations to the genetic aspect of autonomic reactivity: (i) mating between one HR and one LR parent produces offspring with reactivity scores in between the two parents, indicating the trait to be polygenic and its mode of inheritance to be 'intermediate', and (ii) there is no differential effect of male or female parental genes on the autonomic reactivity of the offspring.

The experiment on foster rearing also indicated interesting patterns about the role of rearing by the females. Although the presence of true breeding of the trait was according to the genetic hypothesis, the effect of foster rearing was not as one would expect it to be. According to the hypothesis of environmental influences, one would expect a decrease in reactivity of animals foster reared by LR females, and vice versa. However what was observed in the present study was that foster rearing, of both types, enhanced the reactivity scores in both strains, so much so that the reactivity scores of foster reared animals were higher than those of mother reared animals irrespective of strain. The results once again indicated that the environmental influences, unlike genetic effects, do not follow any specified or predictable pattern.

4.2 *Aggressive dominance*

Although the animals in the first generation experiment were divided into 4 strains, they could also be considered belonging to two groups viz. HA and LA, ignoring their scores on autonomic reactivity. Since the offspring of HA strain scored significantly higher on aggressive dominance than the offspring of LA strain, the results revealed the true breeding nature of the trait.

In addition to the significance of difference between HA and LA strains, two more observations in this experiment were worth noting: (i) except strains (of both types), no other differences were found to be significant, and (ii) when the competition tests conducted were among the animals from identical rearing conditions of different strains, the rearing variable showed a non-significant contribution to the total variance.

When the experiment was repeated, the second generation animal showed the same pattern of aggressivity scores as their parents in the first generation. Although the inheritance pattern came out more clearly in the second generation experiment, a few significant contributions, not observed in the first generation, were also recorded. One such observation was a significant contribution of strain × sex interaction. Although when combined, the HA strain animals scored higher on aggressivity than the LA strain, the difference was more marked in the case of males than females. Looking at the overall results of the two generations, one fact that remarkably stands out is the consistency of inheritance of aggressivity in the strains.

In the first generation experiment, the rearing factor showed a highly significant effect on aggressive dominance. The crowded and the food restricted animals were found to be more aggressive than the normally reared animals. The significance of rearing × sex indicated that the effect of environmental conditions was more marked in the case of males than females. The second generation experiment brought out exactly the same results. A very important aspect of environmental influences is the severity of environmental conditions. The manipulated rearing conditions in the present study were so severe that their influence on aggression is not surprising at all. The food restricted animals not only received lesser food on the average, but also had to compete with each other through the limited access throughout their development. These animals can be expected to show higher aggression than the crowded animals who never had food shortage. Such a difference, however, was not observed. Since the average space available to each crowded animal was half of that for the food restricted animal, 'sensitivity to 'absolute space' could have been the factor enhancing aggressivity in crowded animals. It can be hypothesized that the environmental component of variance may keep on changing, from small to very high, depending upon how mild or strong are the manipulated variables, but the genetic component may always show a consistency.

The experiment involving reciprocal crosses brought out results, as would be expected according to the genetic hypothesis, about a trait which is probably polygenic. The average score on aggressive dominance tests was lowest in the LA strain and highest in the HA strain animals. The scores of the animals produced by reciprocal crosses ranged between the high scoring and the low scoring parents. When all 4 groups were compared, the difference was found to be significant only between the offspring of HA parents and the other 3 groups, and not amongst the 3 groups themselves. The findings suggest that the genes influencing the trait of aggressivity do not seem to indicate any differential inheritance related to sex.

The effect of foster rearing on aggressivity was rather complicated and diverse. The HA strain scored higher than the LA strain. When the mother reared animals (irrespective of strain) were compared with foster reared animals, the obtained F value was non-significant, indicating no effect of fostering. A further analysis, however, revealed the case not to be so. The non-significance of the difference between means was because foster rearing of HA strain by LA females reduced their aggressivity only non-significantly but foster rearing of LA strain by HA females enhanced their aggressivity significantly.

Rearing × sex interaction indicated that the enhancement of aggressivity in foster reared LA strain was significant in the case of males only. It was also observed that crowding and food restriction enhanced aggression significantly only in males. This kind of differential environmental effect on the sexes has enormous significance for the understanding of evolution of behaviour patterns in the two sexes. In natural environment, aggressive behaviour can be beneficial to the males (holding territories or organized into linear dominance systems) but harmful to females. It is possible that there is natural selection for some genes in males to respond more quickly and strongly to environmental pressures than females. In fact, Haug and Mandel (1978) found in mice that the aggression of the two sexes was under separate genetic or hormonal control. Singh *et al* (1984) reported that even in monkeys, the behavioural responses to harsh and hostile environments are more marked in males, even to the extent of resulting in a higher male mortality as compared to females.

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