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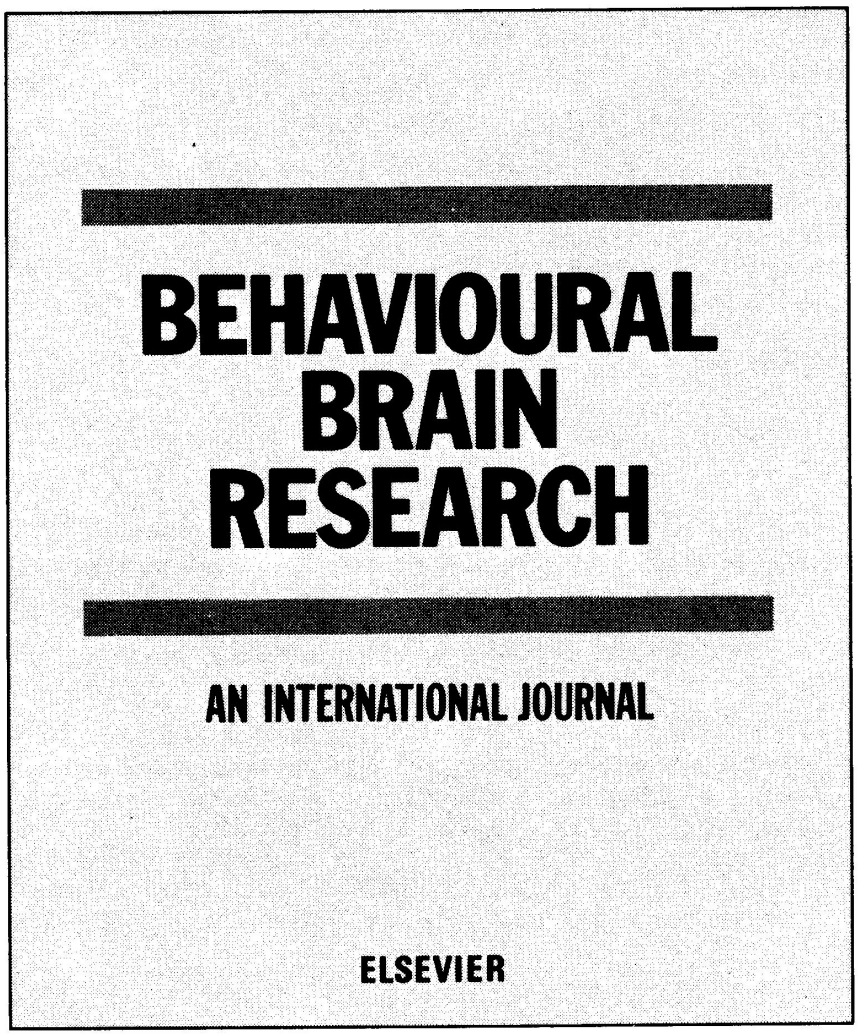

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Genotypic dependency of spontaneous yawning frequency in the rat

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By inbreeding we have obtained two sublines of Sprague–Dawley rats which differ significantly in spontaneous mean yawning frequency (MYF). In generation F21 of the high-yawning (HY) subline MYF was 21.5 yawns/h (y/h) in males and 1.95 y/h in females, at the age of 2 months. In the low-yawning (LY) subline, in generation F16 the MYF was 0.9 y/h in males and only 0.5 y/h in females. During the first 15 days there are no differences in yawning frequency between HY and LY rats. Thereafter yawning increases with age, more steeply in the HY subline. The results of reciprocal crosses between both sublines indicate that the LY character is partially dominant over the HY one.

INTRODUCTION

The mid-twentieth century reviews on yawning by Heusner (1946)[14] and Barbizet (1958)[4] offered adequate coverage of earlier literature on this ubiquitous and apparently trivial behavioral pattern. Apart from a burgeoning of research in the twenties on the physiological mechanisms involved in yawning and on some pathological conditions with which it might be associated, specially by German authors[14], this field has continued to be rather neglected until our day[3]. Even if the mechanisms underlying yawning are far from properly elucidated, and its biological significance is largely ignored, important advances in its understanding may be anticipated, due to the increasing number of research groups, especially among neuropharmacologists, paying attention to this motor act.

In 1955 Ferrari et al.[10] were fortunate enough to come upon a peculiar behavioral syndrome: when dogs were intracerebroventricularly injected with a commercial preparation of adrenocorticotrophic hormone (ACTH), after a latent period of about 30 min, the animals started to yawn and stretch very frequently. This was the first report on pharmacological induction of yawning. Some years later, this Italian group[11,12] extended their observations to other animal species and demonstrated that the stretching-yawning syndrome (SYS) was elicitable both by ACTH and melanocyte-stimulating hormone (MSH). Since in these early experiments they showed the antagonistic effects of atropine and scopolamine on SYS, they

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suggested that a cholinergic mechanism might be involved. More than a decade later several authors demonstrated that other neurotransmitter-related drugs also had yawning-inducing effects[19,28,33] and that most (if not all) of them seemed to involve a cholinergic mechanism, as judged by their susceptibility to muscarinic blocking drugs.

In recent years it has been suggested that elicitation of yawning is mainly the result of an interaction, somewhere in the brain, between inhibitory dopaminergic and excitatory cholinergic influences on the built-in motor program for yawning[15,31,33]. Yawning elicited by low doses of apomorphine and other dopamine (DA) agonists has most generally been interpreted as the result of their selective action on low-threshold DA autoreceptors regulating impulse discharge, synthesis and liberation of the neurotransmitter[7–9,13,19,22,27,29,33]. Nevertheless several authors have postulated that the yawn-inducing effect of low doses of DA agonists is a postsynaptic excitatory effect upon exquisitively sensitive DA2 receptors[20,24]. Higher doses of DA agonists would decrease yawning by acting directly on high-threshold postsynaptic DA yawn-inhibitory receptors[15,19,29,33].

Moreover, there are several other neurotransmitter and hormonal mechanisms known to influence yawning, directly or indirectly[2,5,9,13,16,18,23,24,30,34]. Thus, a rather high number of proteins (enzymes, receptors, etc.) may be involved in the regulation of this behavioral pattern. Therefore, it could be expected that some differences in the level of spontaneous yawning frequency might have a genetic background.

By inbreeding we have developed 2 Sprague–Dawley sublines: one of them yawns spontaneously at a low frequency (LY), the other at a higher level (HY). We describe the evolution of this behavioral pattern along the first year of life in both sublines and the results of reciprocal crosses between them.

MATERIALS AND METHODS

Animals

Both sublines, HY and LY rats, were obtained by inbreeding from Sprague–Dawley rats received in 1981 from the Animal House of the Centro Médico Nacional, I.M.S.S., México.

Animals were housed, 4 rats per cage, under standard conditions, in collective transparent plastic cages (46 × 32 × 20 cm) on wood shavings, with free access to tap water and Purina lab chow, on a 12/12 h light-dark cycle, with dark onset at 19.00 h. All animals were weaned at 30 days and tested for spontaneous yawning frequency when they were 2 months old.

Behavioral observations

As previously described[17] the observations were performed with each animal placed in a transparent glass cylinder (diameter 190 mm, height 100 mm), the floor of which was covered with a sheet of clean filter paper and the top with a plexiglass plate, leaving a 1 cm wide segment open for ventilation. Observations were done regularly from 09.00 to 10.00 h to minimize circadian variation of yawning behavior[1]. Yawning was monitored by 2 trained observers sitting on opposite sides of the table on which the rats were placed. The values ($\pm$ S.D.) referring to spontaneous yawning frequency in the rat, correspond to those monitored in naive animals, during a single observation session, thus avoiding habituation to the experimental conditions.

Statistics

The methods used were mainly nonparametric[25], following the procedure of testing first by analysis of variance (Kruskal–Wallis test) and then by 2-way comparisons (Mann–Whitney U-test), when the former indicated significant differences. The level of significance chosen was $P < 0.05$. Variances were compared with Fisher's F: $S_1^2/S_2^2 \sim F\,(n_1 - 1, n_2 - 1)$.

Estimation of segregating gene pairs

The estimation of the number of segregating gene pairs that influence the character was done according with Bruell's expression (6): $k = (2a^2 + d^2)/4\,(VF2 - VE)$, where k is the number of independently segregating mendelian units for the character under study, VF2 is the F2 variance, a is one half difference between parental strains, d is the deviation of the F1 hybrid from the midpoint between the parental strains and VE is

the environmental variance, estimated as the average variance of the homogeneous parent strains or of the hybrids.

Since rat yawning behavior shows sexual dimorphic expression, males being higher yawners than females[5,16,24], we used yawning frequency in males to calculate the segregating gene pairs.

RESULTS

Spontaneous yawning frequencies in HY and LY sublines

The HY subline began by crossing a male, which yawned spontaneously 22 times per hour when 2-months-old, with one of his sisters. Since F1 males yawned far less than their father, suggesting that HY frequency could be a recessive character, back crosses were set up between him and his F1 daughters. Figure 1 shows the results

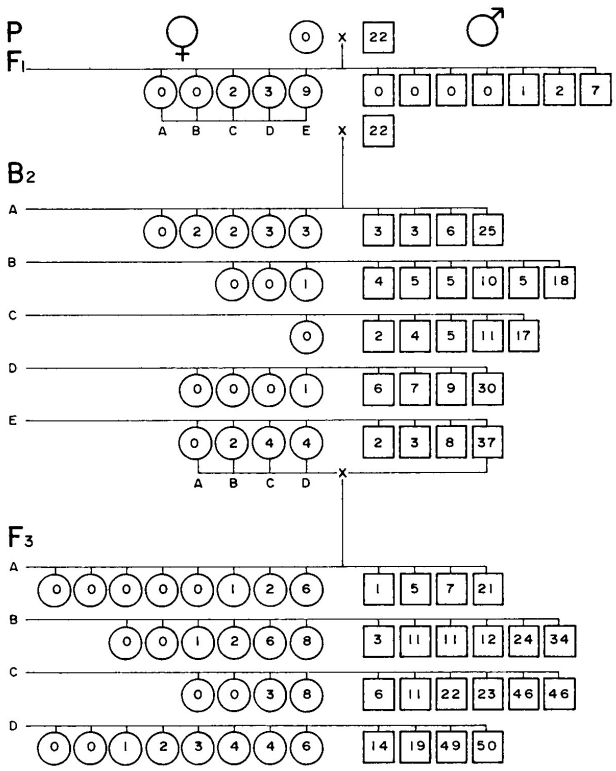

Fig. 1. Origin of the HY Sprague–Dawley subline. Figures inside the symbols (females, ○; males, □) correspond to spontaneous yawning frequency (y/h) monitored when rats were 2 months old. B2: A, B, C, D and E are families from F1: A, B, C, D and E females when crossed with their father. F3: A, B, C and D are descendants from B2: E females when crossed with their indicated brother.

of these crosses and those obtained (F3) from one of B2 families. Descendants were bred by brother-sister mating, selecting the highest yawning male from every litter.

The LY subline corresponds to the original Sprague–Dawley strain, which has been maintained by brother-sister mating, selecting low-yawning animals (males yawning less than 5 times/h) for inbreeding.

Each generation in both sublines, corresponds to descendants obtained from 12–16 crosses.

Beginning with F3 and onwards, the differences in yawning frequency between HY and LY male rats are statistically significant (Kruskal–Wallis test, from F3 to F15, $P < 0.01$; Mann–Whitney U-test for each homologous generation, $P < 0.001$ or less). Yawning behavior of males from the last 10 HY and LY generations is shown in Table I.

The low levels of MYF in LY rats are partly due to lower occurrence of yawning. But if MYF is calculated excluding non-yawning animals, the figures obtained are always below 7 y/h.

Females yawn consistently less than males of their own subline (Kruskal–Wallis, $P < 0.001$ or less). HY females yawn at a slightly higher frequency than LY ones. General mean values for females from F1 to F15 are: HY, 3.2 ± 4.5 y/h ($n = 1112$ animals); LY, 0.5 ± 1.2 y/h ($n = 249$ animals) (Mann–Whitney U-test, one-tailed, $P < 0.001$). When their mean yawning values are calculated excluding non-yawners, the figures obtained are 4.6 y/h for HY females and 2.0 y/h for LY ones. Comparative occurrence of yawning in the females from both sublines was as follows: HY, 62%; LY, 24%.

Heritability estimation

As a first approach to the study of yawning heritability in these 2 sublines 10 crosses between LY (F9) and HY rats (F13) were set up: 5 LY females with 5 HY males in one case, and 5 HY females with 5 LY males in the other. The MYFs of the male rats used in these crosses were as follows: HY = 42.8 y/h; LY = 0.6 y/h. In the case of the females, their brothers had the following MYFs: HY = 34.2 y/h; LY = 2.9 y/h. From these crosses 2 reciprocal F1 populations were

TABLE I

Spontaneous yawning frequencies in the last 10 generations of low and high yawning sublines

HY Males					*LY Males*				
Generation	*n*	*Occ (%)*	*MYF (y/h)*	*S.D. ±*	*Generation*	*n*	*Occ (%)*	*MYF (y/h)*	*S.D. ±*
F12	58	98	28.9	11.5	F7	63	81	5.3	6.8
F13	19	95	28.9	7.4	F8	36	25	2.0	4.0
F14	37	97	24.2	8.5	F9	61	38	2.1	5.6
F15	25	96	25.4	8.1	F10	23	26	1.0	2.1
F16	128	99	31.2	12.9	F11	25	48	1.8	2.3
F17	58	100	20.3	7.6	F12	28	36	1.0	2.4
F18	85	99	26.9	8.4	F13	30	50	1.2	2.1
F19	103	99	35.5	7.4	F14	35	48	2.2	2.6
F20	116	98	22.0	6.1	F15	56	32	1.4	1.8
F21	118	99	21.5	6.2	F16	50	40	0.9	1.7

n, number of males observed (2 months old); Occ, occurrence, percentage of rats that yawned during the standard observation period; MYF, mean yawning frequency (1 h of observation); S.D., standard deviation.
From F3 onwards, the differences between HY and LY homologous generations are highly significant (Mann–Whitney U-test, $P < 0.001$ or less).

obtained (Table II). The MYFs in these sets of animals were: 10.8 y/h (n = 18 males) and 8.9 y/h (n = 22 males) showing no significant difference (Mann–Whitney U-test, $P > 0.05$). These 40 F1 males were crossed with their sisters to obtain the reciprocal F2 generations (n = 84 and 132 males, respectively), which showed no difference in yawning behavior (Mann–Whitney U-test, $P > 0.05$) (Table II). The variance of the MYFs in the F2 generation (pooled data) was significantly larger than the environmental variance (mean of parents and F1 variances) ($F_{215,119} = 6.22$; $P < 0.01$), suggesting that F2 contains a segregating genetic component. No significant differences were found when P1 and P2 variances were compared among themselves ($F_{60,18} = 1.74$). To estimate these values all yawning males from LYF9 and HYF13 were considered. There were no significant differences when the F1 variance (pooled data) was compared with those of P1 or P2 variances ($F_{39,60} = 1.38$); $F_{39,18} = 1.26$). Hence, we calculated a (one half difference between parents yawning values) and d (deviation of the F1 value from the midpoint a) for the reciprocal crosses performed. The following values were obtained: first cross: $a = (42.8 - 2.9)/2 = 19.9$; $d = (19.9 - 8.9) = -11$. Second cross: $a = (34.2 - 0.6/2) = 16.8$; $d = (16.8 - 10.8) = -6$.

With these data, the number of genes involved (k value), estimated with Bruell's expression (see Methods), were calculated. VF2s used were: 292.8 (variance of 132 rats) and 245.8 (variance of 84 rats). VE was the average of both VF1 populations (33.8 + 53)/2) = 43.4. The results obtained were: LY females × HY males, $k = 0.91$ and HY females × LY males, $k = 0.74$, suggesting that the difference between LY and HY rats might be controlled by one segregating unit. Dominance (d/a)[6] was estimated as −0.55 and −0.36, respectively, indicating that the LY character shows a partial dominance over the HY one.

Evolution of yawning during the first year of life

Figure 2 shows the results obtained when 8 males of each subline (HY F14, LY F10) were observed from 10 days old to the age of 12 months. While younger than 20 days old no significant differences in MYF were found between them (Kruskal–Wallis test, $P > 0.05$). For 1-month animals or older the differences in yawning frequencies between LY and HY sublines were highly significant (Kruskal–Wallis test,

TABLE II

Yawning in descendants of HY and LY crosses

Five females of each subline were crossed with 5 males of the other subline. No significant difference was found neither between the reciprocal F1 populations, nor the F2 ones. (Mann–Whitney U-test, $P > 0.05$) For other details see text.

	n	*MYF (y/h)*	*V*	*Occ (%)*
P1 HY F13				
males	19	28.9	54.8	95
females	26	0.8	1.5	54
P2 LY F9				
males	61	2.1	31.4	38
females	21	0.3	0.5	19
LY ♀ × HY ♂				
F1 males	22	8.9	33.8	86
females	25	0.8	0.6	24
F2 males	132	14.9	292.8	81
females	141	1.2	7.2	35
HY ♀ × LY ♂				
F1 males	18	10.8	53	94
females	23	0.5	1.1	26
F2 males	84	18.3	245.8	76
females	106	2.2	18.1	51

N, number of animals (2 months old); MYF, mean yawning frequency (1 h of observation); *V*, variance; Occ, percentage of rats which yawned during the observation period; P1 and P2, parents; F1 and F2, first and second generations.

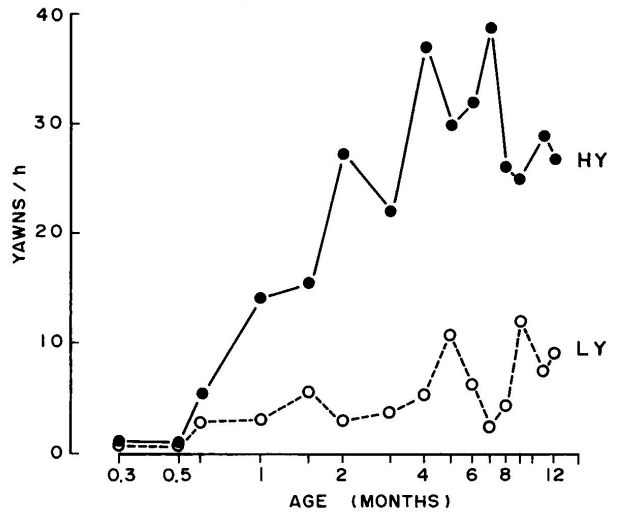

Fig. 2. Evolution of yawning behavior along the first year of life. Average curves obtained with 8 HY and 8 LY male rats. Differences between both sublines from 1 month old onwards: Kruskal–Wallis test, $P < 0.01$; Mann–Whitney U-test, $P < 0.001$ or less for each age.

DISCUSSION

Research in yawning behavior has moved during the past decade from a rather neglected position to become a field of rapidly growing interest, particularly for neuropharmacologists. With the aim of developing better experimental subjects for the physiological analysis of this behavior, we have endeavoured to obtain inbred genetic sublines of Sprague–Dawley rats with high and low spontaneous yawning rates.

The increase in yawning activity in HY males was observed from the very beginning of inbreeding, the differences between homologous generations of HY and LY rats being highly significant from F3 onwards.

Yawning activity in HY females is slightly but significantly higher than in LY females. In both sublines females yawn much less than males, confirming the sexual dimorphic character of this behavior[5,16,24].

We feel it must be stressed that the quantitative data on yawning here reported have been obtained under very strictly standardized observational conditions (see Methods), which have been maintained during almost 8 years. Yawning frequency in HY animals is more susceptible than in LY rats to changes in the conditions under which the animals are observed: it decreases during social interaction with other littermates when animals are placed in collective cages; it increases, due to habituation to being placed singly in novel environments, when observation sessions are repeated at daily intervals[32]; it also has an important circadian variation[1].

Yawning activity increases with age. Young and adult rats yawn at a higher rate than infant animals. It is well known that at puberty there is an important increase in testosterone levels in male rats[21] and it has been demonstrated that the administration of this sex hormone promotes an important increase in yawning frequency both in female rats and castrated males[5,16]. However, we have not found any difference in the serum levels of testosterone between HY and LY rats, so we

are inclined to discard androgenic hormonal factors as contributors to the difference in yawning rates observed between these two sublines (Eguibar et al., unpublished results).

Biometrical analysis of non-segregating populations (P1, P2 and F1) after reciprocal genetical crosses between animals of the HY and LY sublines, suggested that one pair of genes is involved in the difference between these sublines and that the LY (P1) subline is partially dominant over the HY (P2) one. Since no differences were found between reciprocal F1 or F2 generations, maternal factors may be excluded.

Yawning behavior is subject to important dopaminergic (inhibitory) and cholinergic (excitatory) influences[15,33]. If tonic dopaminergic inhibitory control diminishes, yawning frequency increases. The same happens with an increase in cholinergic activity. Thus, on a still rather loose conjectural basis, we think that HY rats may have a higher tonic cholinergic activity than LY animals. An increase in cholinergic tone in HY rats could be understood as a direct and general effect, intrinsic to the cholinergic system as a whole, or an indirect and more particular phenomenon, resulting from a decrease in tonic DA inhibitory activity, and therefore restricted only to cholinergic pathways subject to dopaminergic restraining control. We do not yet have a definite choice between these alternative hypothetical possibilities, which are under current experimental scrutiny with pharmacological tools.

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