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Hereditary changes of male aggressiveness after gamma irradiation of mouse spermazozoa with 600 R

by

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Erbliche Veränderungen der Aggressivität männlicher Mäuse nach Gamma-Bestrahlung der väterlichen Spermatozoen mit 600 R

S y n o p s i s : Männliche F_1-Nachkommen von bestrahlten NMRI-Labormäusen, deren Spermatozoen einer Gammabestrahlung von 600 R ausgesetzt worden waren, wurden mit männlichen NMRI-Mäusen unbestrahlter Väter für die Dauer von 24 Stunden zu Paaren zusammengesetzt. Die von bestrahlten Vätern abstammenden F_1-Männchen zeigten eine durchschnittlich höhere Aggressivität als die von unbestrahlten Männchen abstammenden Tiere, wobei das Maß der Aggressivität durch Auszählung der am Schwanz und Körper vorgefundenen Läsionen bestimmt wurde. Die Aggressivität der aus bestrahlten Spermatozoen hervorgegangenen F_1-Männchen war jedoch nicht einheitlich: Neben schwach-aggressiven Männchen, die etwa dasselbe Aggressionsniveau wie ihre unbestrahlten Kontrahenten besaßen, gab es hoch-aggressive F_1-Männchen, von denen die Hälfte sich als steril erwies. Nach Weiterzucht mit unbestrahlten NMRI-Weibchen zeigten zwar alle Söhne der F_1-Männchen eine durchschnittlich geringere Aggressivität als ihre unbehandelten Kontrahenten, was auf Rezessivität für hohe Aggressivität hinweist, doch war die Aggressivität der von schwach-aggressiven F_1-Vätern abstammenden Männchen signifikant stärker reduziert als die Aggressivität der von hoch-aggressiven F_1-Vätern abstammenden Männchen. Es wird angenommen, daß hohe Aggressivität hauptsächlich auf strahleninduzierten Translokationen beruht.

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*) Dedicated to Nobel-prize winner Prof. Dr. Dr. Dr. h.c. mult. K. Lorenz on the occasion of his 75th birthday.

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Introduction:

An increase in male aggressiveness found in F_1 mice (*Mus musculus domesticus*) derived from spermatozoa irradiated with 600 R of gamma rays was presumed to be of genetic nature (SCHRÖDER, 1977). It was proposed to investigate the hereditary basis of altered aggressiveness by selective breeding of low and high aggressive males as has already been shown for offspring of irradiated cichlid fish (HOLZBERG and SCHRÖDER, 1975). The present paper deals with this topic and thus completes the first communication on radiation-induced increases in aggressiveness in the house mouse.

Material and Methods:

The Neuherberg NMRI strain used in these experiments is kept as a specific-pathogen free mouse strain as described elsewhere (SCHRÖDER, 1977). Males were irradiated with 600 R of Cs-gamma rays at a dose-rate of 30 R/min, measured by a Victoreen-dosimeter. During exposure, only the pelvic region was irradiated directly while the head and the front part of the trunk were shielded with 10 - 12 mm of lead. To use irradiated spermatozoa for fertilization of the ova, irradiated and sham-treated males were mated to untreated NMRI females during the first week after exposure (OAKBERG, 1957). 10 to 12 week old male F_1 offspring of both series were put together for 24 hours (from 10 a.m. to 10 a.m. next day) in Macrolon boxes, 25 x 20 x 14 cm. Each box contained one pair of males, one irradiated (I) and one control (C) F_1 male. F_1 males selected for further breeding were mated to untreated NMRI females thus producing an outcross generation with an average of one quarter of the replicates of the irradiated genome. The outcross males were divided into two groups according to the aggressiveness of their F_1 fathers, *i. e.* one group of outcross males was derived from high aggressive I - F_1 males while the second group stemmed from low aggressive I - F_1 fathers. For the determination of aggressiveness, the outcross males were handled in the same manner as their F_1 ancestors. Because all the males were isolated from weaning, the two males of a given pair were unknown to each other and inexperienced in fighting before being tested. After separation of the paired males, the number of wounded sites ("sores") on the tail and on other parts of the body was counted according to the procedure of COLLINS (1970) and LEVINE *et al.* (1965). The male which delivered more and received fewer bites than his opponent was designated the "winner", while the "loser" received more and delivered fewer bites than his opponent. In order to prevent home-cage effects, both males were put together into a new box simultaneously. Although weight differences between the two males of a pair were minimized as far as possible and most of the pairs were of equal weight at the beginning of the test, some weight differences had to be tolerated. The weight differences between the two males of a pair varied between 0 and 3 g for F_1 and between 0 and 1.7 g for outcross males, the absolute values of weights ranging from 37 to 47 g for F_1 and from 29 to 37 g for outcross males. As shown by statistical calculations, weight differences did not influence the success of fighting under the conditions of the experiment. Therefore, any detectable differences between the aggressiveness of outcross males derived from either high or low aggressive F_1 fathers would be indicative of the hereditary basis of altered aggressiveness.

Results:

1. F_1 males:

The effect of paternal irradiation on the agonistic behaviour of F_1 males was already described in a previous paper (SCHRÖDER, 1977). However, it was not yet determined that the aggressiveness did not increase in a uniform way: 13 out of 34 F_1 males exhibited an extreme high level of aggressiveness, 6 were of lower aggressiveness, and the remaining 15 males derived from irradiated spermatozoa were of median aggressiveness (Table 1). Only the high and low aggressive I - F_1 males which differed significantly from each other were selected for further breeding, whereas the 15 males with median aggressiveness remained unmated. Among the 13 high aggressive F_1 males, 6 males were unsuccessful in producing offspring after mating to untreated NMRI females, thus constituting a fourth group of sterile males which differed significantly only from the low aggressive group. The median aggressive group was significantly different from the high aggressive group but did not differ significantly from the mean of the low aggressive F_1 males (t-test, $p < 0.05$). The overall aggressiveness of all I - F_1 males as compared to their C - F_1 opponents was significantly higher as described in more detail in the previous paper (SCHRÖDER, 1977).

Table 1: Agonistic behaviour of NMRI F_1 (I) males (600 R of Cs-gamma rays to paternal spermatozoa) directed to untreated (C) NMRI males.

Characterization of the I-F_1 males	Number	Proportion of winners (W), losers (L), and ties (T)	Wounded sites ("sores") on the tails (Mean ± S.E.)	
			Bites received	Bites delivered
Selected for further breeding; high aggressive (HA)	7	7 W, 0 L, 0 T	0.00	28.00 ± 5.98 1)3)
Selected for further breeding; low aggressive (LA)	6	1 W, 2 L, 3 T	3.17 ± 2.40	0.50 ± 0.50 1)2)
Sterile; high aggressive	6	5 W, 0 L, 1 T	0.00	13.83 ± 4.11 2)
Not mated; median aggressive	15	11 W, 1 L, 3 T	0.15 ± 0.09	4.07 ± 1.01 3)

1)2)3) Significant differences between high, low and median aggressive F_1 males.

2. Outcross males:

The outcross males were then divided into two groups according to their derivation from either high or low aggressive F_1 fathers (Table 2). When one compares their agonistic behaviour with that of their control (C) opponents, both types of outcross males were found to be less aggressive than the corresponding opponents. However, in the case of outcross males derived from high aggressive F_1 fathers (part A of Table 2), a significant level (95 %-confidence limits, standard chi-square test) was reached only

Table 2: Agonistic behaviour of NMRI outcross (I) males (F_1 male x untreated female) directed to untreated (C) NMRI males.

A. I-males derived from high aggressive F_1 fathers.

Criterion of agonistic behaviour	Series	Number*	Mean ± S.E.	95 %-Confidence limits of the mean	χ^2 p
Males with wounded tails	I	17	0.39 ± 0.07	0.25 - 0.53	0.53
	C	13	0.30 ± 0.07	0.16 - 0.44	0.45
Wounded sites ("sores") on the tails	I	120	2.73 ± 0.73	1.00 - 4.46	8.45
	C	79	1.80 ± 0.63	0.51 - 3.09	0.0035
Males with lesions other than wounded tails	I	8	0.18 ± 0.06	0.06 - 0.30	0.47
	C	11	0.25 ± 0.07	0.11 - 0.39	0.49
Males with both types of lesions	I	10	0.23 ± 0.06	0.11 - 0.35	0.22
	C	8	0.18 ± 0.06	0.06 - 0.30	0.65
Wounded males with any lesion	I	25	0.57 ± 0.08	0.41 - 0.73	0.02
	C	24	0.55 ± 0.08	0.39 - 0.71	0.89
Males without any detectable lesion	I	19	0.43 ± 0.08	0.27 - 0.59	0.026
	C	20	0.45 ± 0.08	0.29 - 0.61	0.87

*) 44 NMRI males of each series tested.

B. I-males derived from low aggressive F_1 fathers.

Criterion of agonistic behaviour	Series	Number**	Mean ± S.E.	95 %-Confidence limits of the mean	χ^2 p
Males with wounded tails	I	24	0.75 ± 0.08	0.58 - 0.92	2.63
	C	14	0.44 ± 0.09	0.25 - 0.63	0.11
Wounded sites ("sores") on the tails	I	169	5.84 ± 1.34	3.03 - 8.65	98.99
	C	29	0.91 ± 0.27	0.34 - 1.48	$< 10^{-10}$
Males with lesions other than wounded tails	I	1	0.03 ± 0.03	0.00 - 0.09	4.50
	C	7	0.33 ± 0.07	0.07 - 0.37	0.035
Males with both types of lesions	I	18	0.56 ± 0.09	0.37 - 0.75	7.35
	C	5	0.16 ± 0.07	0.01 - 0.31	0.007
Wounded males with any lesion	I	25	0.78 ± 0.07	0.63 - 0.93	0.35
	C	21	0.66 ± 0.09	0.47 - 0.85	0.56
Males without any detectable lesion	I	7	0.22 ± 0.07	0.07 - 0.37	0.88
	C	11	0.34 ± 0.09	0.15 - 0.53	0.34

**) 32 NMRI males of each series tested.

for the different number of wounded sites on the tails as compared to the controls. Males derived from low aggressive F_1 fathers differed significantly more often from their opponents: More wounded sites on the tails were observed in I than in C males, more C males were found with lesions other than wounded tails, and more I than C males exhibited both types of lesions (part B of Table 2). Accordingly, the reduction of aggressiveness in comparison to the corresponding opponents was found actually to be more pronounced in I males derived from low aggressive F_1 males than in I males sired by high aggressive F_1 fathers. The comparison of the agonistic behaviour of both types of outcross males by use of the I/C values also reflects this situation (Table 3). The differences of the I/C values for outcross males derived either from high or low aggressive F_1 fathers reached significant levels ($p < 0.05$) for wounded sites on the tails and for the proportion of winners/losers (chi-square test for two independent samples; SIEGEL, 1956; SACHS, 1973). Thus, outcross males sired by low aggressive F_1 males received significantly more bites and were more often losers in the sense as defined above than did outcross males sired by high aggressive F_1 males. In other words, outcross males derived from high aggressive F_1 fathers were more aggressive than those from low aggressive F_1 fathers.

Table 3: Comparison of the agonistic behaviour of outcross males derived from either high or low aggressive F_1 fathers.

Criterion of agonistic behaviour	I/C high aggressive *	I/C low aggressive *
Males with wounded tails	1.31	1.71
Wounded sites ("sores") on the tails	1.52 [1)]	5.83 [1)]
Males with lesions other than wounded tails	0.73	0.14
Males with both types of lesions	1.25	3.60
Wounded males with any lesions	1.04	1.19
Males without any detectable lesion	0.95	0.64
Winners / Losers	0.83 [2)]	0.30 [2)]

*) I/C calculated as: $\frac{\text{Number of scores (males) in the irradiated series}}{\text{Number of scores (males) in the control series}}$

1)2) Significant differences between outcross males derived from high and low aggressive F_1 fathers

Discussion:

The question arises of how to explain the lower aggressiveness of outcross males as compared to their opponents in view of the fact that they all were derived from F_1 males exhibiting an overall higher aggressiveness than untreated NMRI male mice. Keeping in mind that 6 out of 13 high aggressive F_1 males were sterile (Table 1), almost 50 % of high aggressive males could not be tested for the possible inheritance of increased aggressiveness. Otherwise, sterility and semi-sterility are well-known phenomena explained by heterozygosity for translocations caused by radiation-induced chromosome breaks (*e. g.* ADLER, 1978; CACHEIRO *et al.*, 1974; HERTWIG, 1940; SEARLE *et al.*, 1974). Therefore, one can imagine that heterozygous carriers of radiation-induced chromosomal rearrangements may contribute considerably to the increase in aggressiveness found in the irradiated F_1. Thus, only semisterile F_1 males might be included in the group of 7 high aggressive F_1 males selected for further breeding. If an increase in aggressiveness is associated with heterozygosity for translocations, only less severe translocations can be tested by further breeding. Despite this handicap, the remaining F_1 males selected for further breeding gave clear evidence that high aggressive F_1 males sired more aggressive sons than did low aggressive F_1 males. There is no doubt that radiation-induced alterations in aggressiveness have a genetic origin. Differences in juvenile environment resulting from smaller litter-sizes of F_1 males derived from irradiated spermatozoa (SCHRÖDER, 1977) have only a minor, if any, influence on agonistic behaviour.

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