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The video tape analysis of agonistic behaviour of male house mice (*Mus musculus domesticus*)

by

Johannes Horst SCHRÖDER *)

(Institut für Biologie, Gesellschaft für Strahlen- und Umweltforschung, Neuherberg bei München, and Institut für Zoologie der Universität Innsbruck)

Die Analyse des agonistischen Verhaltens der männlichen Hausmaus (*Mus musculus domesticus*) durch Auswertung von Videoaufnahmen

S y n o p s i s : Es wird eine Methode zur quantitativen Erfassung des agonistischen Verhaltens männlicher Mäuse vorgestellt, wobei jeweils zwei Tiere gleichen Gewichtes und ohne soziale Erfahrung seit der Entwöhnung für die Dauer von 24 Std. Gelegenheit bekommen, miteinander zu kämpfen. Die ersten 10 Min. der Auseinandersetzung werden mit einer AKAI-Videokamera aufgenommen. Das agonistische Verhalten jedes einzelnen Männchens wird anschließend durch mehrmaliges Abspielen desselben Videobandes mit Hilfe eines Stopprechners getrennt ausgewertet, wobei Häufigkeit und Dauer folgender Verhaltensweisen registriert werden: Explorationsverhalten (Herumlaufen, Aufrichten, Schnüffeln an Gegenständen, Springen, Graben), Putzen, Inaktivität (Immobilität, Erstarren), Schwanzrütteln, Kontakt zum Kontrahenten (Beschnuppern, Putzen des Partners, Überkriechen), Verfolgen, Fliehen, Angreifen, Kampf und Verteidigung. 24 Std. nach dem Zusammensetzen werden die Bisse am Schwanz und am Körper ausgezählt. Die Maus, welche weniger Bißwunden als ihr Kontrahent zeigt, wird zum "Gewinner" erklärt, der Partner mit mehr Bißwunden zum "Verlierer". Die Gewinner wiesen eine größere Häufigkeit und/oder Dauer folgender Verhaltensweisen auf: Explorations- und Putzverhalten, Schwanzrütteln, Verfolgen und Angreifen, während Flucht- und Defensivverhalten im Vergleich zu den Verlierern vermindert waren. Die Methode eignet sich zur Erfassung mutativer Veränderung des angeborenen agonistischen Verhaltens der Hausmaus nach ancestraler Behandlung mit Mutagenen.

*) Author's address: Univ.-Prof. Dr. J.H. Schröder, Institut für Biologie, Gesellschaft für Strahlen- und Umweltforschung, Ingolstädter Landstraße 1, D-8042 Neuherberg, BRD.

Introduction:

According to a proposal of LEVINE *et al.* (1965) and COLLINS (1970), the success of fighting male mice can be determined by counting the number of wounded sites ("sores") on the tails, feet and trunk. This procedure was used successfully to examine the mutagenic effect of ionizing radiation on the aggressiveness of male offspring after exposure of paternal spermatozoa to 600 R of gamma rays (SCHRÖDER, 1977; 1979). To check the reliability of this method and to improve the detailed analysis of aggressiveness, the first 10 minutes of a total of 24 hours in which a naive male mouse was allowed to encounter a naive male opponent were analysed by the use of video recordings. Thus, the agonistic behaviour of each of the two males could be determined separately by repeatedly playing the video tape. The results of the first 10 minutes of agonistic encounters were then compared with the number of wounded sites on both opponents. The male which received fewer and delivered more bites than his opponent was designated the winner. The frequency and duration of ten behavioural traits of winners and losers were compared.

Material and Methods:

Untreated NMRI males, 10 - 12 weeks old when tested for aggressiveness, were used in this experiment. The Neuherberg NMRI mouse strain is kept under specific-pathogen free conditions as described elsewhere (SCHRÖDER, 1977). Because all the males were kept isolated since weaning, the two males of a given pair were unknown to each other and unexperienced in fighting before being tested. After 24 hours of agonistic encounters, the two opponents were separated and the number of wounded sites ("sores") on the tail, trunk, and feet was counted. During the first 10 minutes after putting them together, video records were taken of their encounters with an AKAI B/W video camera. In oder to distinguish both males from each other for the subsequent analysis of the video tape, one male of each pair was marked before the fight with a black spot on the back. For each of the two males, the frequency (F) and duration (D), measured in 0.01 minutes, of ten behavioural traits were determined with the aid of an event recorder (PEISELER Stopprechner). The ten behavioural traits scored for quantification were as follows: Exploratory behaviour (E), including walking ("ambulation"), rearing, sniffing, jumping, and digging; autogrooming (A); inactivity (I), including immibility and freezing reactions; tail rattling (T); contacts to the partner (C), including sniffing, allogrooming and attempts to cover the opponent with the body; following the partner (Fo); fleeing from the partner (Fl); attacking the partner (At); fights (F), both oppenents swirling around each other without recognizing any detail; and defensive behaviour (D) as described elsewhere (EIBL-EIBESFELDT, 1950; 1958). To prevent home-cage effects, both males were put together into a new Macrolon box (25 x 20 x 14 cm) simultaneously. Although weight differences between the two males of a pair were minimized as far as possible and most of the 19 pairs under investigation were of equal weight at the beginning of the agonistic encounter, small weight differences could not be excluded. Statistical treatment showed, however, that they did not influence the success of fighting, *i. e.*, there was no correlation between the weight of the opponents and their success in fighting. The sign test for matched pairs was used for statistical comparisons between the paired males (SIEGEL, 1956).

Results:

All males of the 19 NMRI pairs tested for aggressiveness were divided into winners or losers according to their success in fighting after 24 hours. The winners (W) delivered more bites than they received from their opponents, and the losers (L) received more bites than they delivered. As seen in the upper part of Table 1, significantly more losers exhibited wounded tails and number of wounded sites ("sores") on the tail and trunk than did winners. This agrees with the significant differences for the ten behavioural traits between losers and winners (lower part of Table 1): Both frequency and duration of T, Fo, and At, and only duration of E and A were higher for winners than for losers, whereas lower values for both frequency and duration of Fl and D were found for winners. No significant differences between winners and losers were found both for frequency and duration of I, C, and F. Because the duration of E and A was significantly higher for winners and I was slightly but insignificantly reduced for winners, losers seem to have a lower locomotory activity than winners.

Discussion:

The present findings gave clear evidence that the previously used method of counting the wounded sites on the tail and other parts of the body presents a reliable procedure for determining the aggressiveness of male mice. This is because the results after 24 hours of agonistic encounters are confirmed by a more detailed analysis of the fighting behaviour during the first ten minutes as determined by analysis of video records. Winners seem to exhibit more locomotory activity than losers as indicated by higher levels of exploratory behaviour and autogrooming and lower inactivity. On the other hand, higher levels of tail rattling, following and attacks with the corresponding reduction of fleeing and defensive behaviour reflect directly the higher success of winners during agonistic encounters. Thus, both the determination of wounded sites and the analysis of agonistic encounters from video records can be used to detect hereditary changes of quantitative aggressive behaviour after ancestral treatment with mutagens.

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Table 1: Agonistic behaviour of NMRI control males directed to untreated NMRI males [1] (Analysis of video records)

Criterion of agonistic behaviour			Winners (W)				Losers (L)				W/L
			No.	$\bar{x}$	s_x	$\overline{s_x}$	No.	$\bar{x}$	s_x	$\overline{s_x}$	
Males with any lesion [2]			11	0.58	0.51	0.12	17	0.89	0.32	0.07	0.65
Males withuout any detectable lesion			8	0.42	0.51	0.12	2	0.11	0.32	0.07	4.00
Males with wounded tails			5	0.26	0.45	0.10	14	0.74	0.45	0.10	0.36*
Males with other lesions than wounded tails			10	0.53	0.51	0.12	15	0.79	0.42	0.10	0.67
Males with both types of lesions (wounded tails plus any additional lesion)			4	0.21	0.42	0.10	12	0.63	0.50	0.11	0.33
Wounded sites ("sores") on the tails			22	1.16	2.85	0.65	198	10.42	12.25	2.81	0.11*
Wounded sites ("sores") on the trunk			7	0.37	0.60	0.14	25	1.32	1.38	0.32	0.28*
Wounded sites ("sores") on the feet			9	0.47	0.84	0.19	13	0.68	1.11	0.25	0.69
Analysis of video records (10 min of observation time)	Exploratory Behaviour (E)	F [3]	1271	66.89	21.65	4.97	1161	61.11	17.17	3.94	1.10
		D [4]	7256	381.89	138.71	31.82	6428	338.32	90.86	20.84	1.13*
	Autogrooming (A)	F	234	12.32	5.67	1.30	161	8.47	5.74	1.32	1.45
		D	957	50.37	34.70	7.96	722	38.00	36.04	8.27	1.33*
	Inactivity (I)	F	366	19.26	15.10	3.46	447	23.53	11.11	2.55	0.82
		D	1826	96.11	75.77	17.38	2690	141.58	82.25	18.87	0.68
	Tail rattling (T)	F	862	45.37	28.29	6.49	484	25.47	20.28	4.65	1.78*
		D	1445	76.05	48.16	11.05	877	46.16	37.77	8.67	1.65*
	Contacts to the partner (C)	F	1052	55.37	23.63	5.42	1025	53.95	21.91	5.03	1.03
		D	3235	170.26	87.16	20.00	3585	188.68	100.14	22.97	0.90
	Following the partner (Fo)	F	6.31	33.21	19.46	4.46	268	14.11	20.89	4.79	2.35*
		D	1454	76.53	48.51	11.13	796	41.89	60.64	18.50	1.83*
	Fleeing from the partner (Fl)	F	192	10.11	17.62	4.04	516	27.16	21.23	4.87	0.37*
		D	497	26.16	61.39	14.08	1222	64.32	54.22	12.44	0.41*
	Attacking the partner (At)	F	996	52.42	44.46	10.20	253	13.32	12.97	2.98	3.94*
		D	1244	65.47	50.94	11.69	192	10.11	12.40	2.84	6.48*
	Fights (F)	F	778	40.95	21.67	4.97	817	43.00	20.66	4.74	0.95
		D	1856	97.68	62.12	14.25	1911	100.58	63.03	14.46	0.97
	Defensive Behaviour (D)	F	208	10.95	14.38	3.30	903	47.53	46.40	10.64	0.23*
		D	316	16.63	25.04	5.74	2154	113.37	107.84	24.74	0.15*

1) 19 pairs of NMRI control males tested ($\bar{x}$, mean; s_x, standard deviation; s_x, standard error of the mean)
2) after 24 hours of fighting
3) F, frequency
4) D, duration as measured in 0.01 minutes
*) significant differences between W and L ($p < 0.05$)

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