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COHABITATION WITH A FEMALE INDUCES THE SAME LEVEL OF AGGRESSION AS ISOLATION IN OF1 MALE MICE

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The isolation or individually-housed paradigm is one of the most frequently used in male mice, although animals submitted to this procedure show alterations in some behavioral and physiological variables. In this study, the level of aggression induced by isolation was compared with that induced by cohabitation with a female in OF1 male mice. After a period of four weeks of either isolation or cohabitation with a female, male mice were confronted with a non-aggressive opponent in a neutral area for 10 minutes. Encounters were videotaped and the behavior of animals was analyzed. The results showed that both paradigms induced the same level of aggression in this strain. Furthermore, no differences in other behaviors were found. This study suggests that cohabitation with a female is not only a more natural but also a valid paradigm to induce aggression in OF1 male mice.

La cohabitación con una hembra induce el mismo nivel de agresión que el aislamiento en ratones macho OF1. Uno de los paradigmas más utilizados para inducir agresión en ratones machos es el aislamiento o alojamiento individual, a pesar de las alteraciones fisiológicas y conductuales que produce. En este estudio, se ha comparado el nivel de agresión inducido por aislamiento con el inducido mediante cohabitación con una hembra en ratones machos OF1. Tras cuatro semanas de aislamiento o cohabitación con una hembra, se confrontó a los ratones machos con un oponente no agresivo en una área neutral durante 10 minutos. Se registraron los encuentros y se analizó la conducta de los animales. Los resultados muestran que ambos paradigmas inducen el mismo nivel de agresión en esta cepa. Además, no se observaron diferencias en otras conductas. Este estudio sugiere que el paradigma de cohabitación con una hembra, siendo más natural que el aislamiento, es igualmente válido para inducir agresión en ratones machos OF1.

Different animal models are used in the laboratory to induce aggression in male rodents (Eichelman, Elliot and Barchas, 1981). The most commonly used in mice is the isolation paradigm, in which animals are individually housed for a period of some weeks before being confronted with another male. During this period, animals maintain visual, auditory and olfactory communication with other conspecifics in the same room. The level of aggressiveness increases with the duration of the isolation although it varies with

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strain and age (Valzelli and Garattini, 1968; Eleftheriou, Bailey and Denenberg, 1974; Eichelman et al., 1981; Siegfried, Alieva, Oliverio and Puglisi-Allegra, 1981; Puglisi-Allegra and Cabib, 1985).

It is well known that isolation has effects not only on aggression but also on other kinds of behavior, hormones and neurotransmitter systems (Valzelli, 1973; Valzelli and Bernasconi, 1979; Brain and Benton, 1983; Hilakivi, Ota and Lister, 1989; Cabib and Puglisi-Allegra, 1990). All these changes have been described as the «isolation syndrome» (Valzelli, 1973, 1981) which may possibly influence the effect of pharmacological treatments on aggression in pre-clinical studies.

In other rodent species such as rats the cohabitation with a female paradigm has been commonly used to induce aggression. However, the studies employing this paradigm in mice are scanty (Brain, Homady, Castaño and Parmigiani, 1987; Parmigiani, Mainardi, Brain, Haug and Brunoni, 1989; Yoshimura and Kimura, 1991). Few studies have compared the level of aggression induced by isolation and by cohabitation with a female (Brain, Benton and Bolton, 1978; O´Donnell, Blanchard and Blanchard, 1981; Jones and Brain, 1987; Zochi, Cabib and Puglisi-Allegra, 1994; Moya-Albiol, Salvador, Martínez-Sanchis and Costa, 1995) and the results obtained are different depending on the strain.

In order to study the possibility of using a more natural paradigm to induce aggression than the «isolation» model, the present study compared the level of aggression induced by isolation which that induced by cohabitation with a female in OF1 male mice.

Materials and methods

Subjects and Housing

Ninety nine commercially-acquired (IFFA CREDO, Lyon, France) OF1 albino mice (86 males and 13 females) arrived at the laboratory at 42 days of age. One group of males (n = 60) was housed in groups of five in opaque plastic cages measuring 24.5 x 24.5 x 15 cm (PANLAB, Barcelona, Spain) and used as non-aggressive anosmic opponents. They were rendered anosmic 24 hours before agonistic encounters. Each mouse was lightly anaesthetized with ether and then 25 ul of a 4% aqueous solution of zinc sulphate was introduced into each nostril. They were inverted shortly afterwards to prevent their swallowing the toxic solution (Alberts and Galef, 1971; Brain, Goldsmith, Parmigiani and Mainardi, 1982). Another group (n = 26) was used as experimental animals. Half of them were individually housed in transparent plastic cages measuring 24 x 14 x 13 cm (LETICA S.A. Barcelona, Spain) and the other half were paired with a female in an identical cage. All animals lived under a reversed lighting schedule (white lights on from 20:00 to 08:00 hr local time) and were maintained at 18-21ºC. Food and water were supplied ad libitum.

Agonistic encounters

Four weeks after living in isolation or cohabiting with a female each experimental male was submitted to an agonistic encounter with an anosmic opponent in an all-glass neutral cage (60 x 33 x 30 cm) located in an observation room. They were initially separated by a plastic partition for an adaptation period of one minute and, after removal of the partition, they interacted for ten minutes. All encounters were carried out during the dark period (between the third and sixth hour of the dark phase), under a red light from a 25 watt bulb. The sawdust substrate was changed and the cage cleaned after each encounter.

Behavioral analysis

Encounters were video-taped using a Panasonic NV camera, a Panasonic NV-770

video and a Sony Trinitron monitor. The behavioral analysis involved assessment of the behavior of the experimental male during the agonistic encounter. Tapes were analyzed using an ethologically-based methodology assisted by a microprocessor (Brain, McAllister and Walmsley, 1989). The computer program enabled the assessment of total duration, frequency and latency of eleven broad categories of behavior. Each category consisted of groups of well-defined specific acts, postures or elements of behavior (Martínez, Castaño, Simón and Brain, 1986) that are given in Table I.

Table 1
Functional categories of behavior used in intermale agonistic encounters in mice

Categories of Behavior	Constituent Elements
BODY CARE	Abbreviated groom, Self groom, Wash, Shake, Scratch
DIGGING	Dig, Kick dig, Push dig
NON SOCIAL EXPLORATION	Explore, Rear, Supported rear, Scan
EXPLORE FROM A DISTANCE	Approach, Attend, Head orient, Stretched attention
SOCIAL INVESTIGATION	Crawl over, Crawl under, Follow, Groom, Head groom, Investigate, Nonse sniff, Sniff, Push past, Walk around
THREAT	Aggressive groom, Sideways offensive, Upright offensive, Tail rattle
ATTACK	Charge, Lunge, Attack, Chase
AVOIDANCE/FLEE	Evade, Flinch, Retreat, Ricochet, Whell, Startle, Jump leave, Wall clutch
DEFENSIVE/SUBMISSIVE	Upright defensive, Upright submissive, Sideways defensive
SEXUAL	Attempted mount, Mount
IMMOBILITY	Squat, Cringe

Statistical Analysis

Data of the total duration, frequency and latency of each broad behavioral category exhibited by isolated or cohabiting with a female male mice were subjected to appropriate paired comparisons using Mann-Whitney «U» test. A probability level less than 0.05 was considered as significant.

Results

The results of the behavior shown by isolated or cohabiting with a female mice are given in Table II. No defense, avoidance, immobility or sexual behaviors were recorded in either group. As can be seen in Table II no significant differences were found between groups in any of the behavioral categories recorded.

Discussion

The results indicate that the same level of intermale aggression (attack and threat) is induced either by isolation or cohabitation with a female although the level of aggression was low and the latency to first aggression long in comparison with previous studies with the isolation paradigm (Martínez, Salvador and Simón, 1994). Our results agree with other studies which found that male mice cohabiting with a female showed a similar level of intermale aggression to isolated ones, although higher or lower levels of aggression have also been reported in males cohabiting with a female. It has been suggested that one of the reasons for the different results obtained could be the genetic differences. In this sense, similar levels of aggression have been reported in TO and Swiss albino outbred strains (O´Donnell et al., 1981; Brain and Benton, 1983; Jones and Brain, 1987) with both paradigms, while a higher level of aggression induced by isolation in DBA/2 and C57BL/10 strains

(Jones and Brain, 1987; Zochi et al., 1994), and a higher level of aggression induced by cohabitation in TO and C57BL/6 strains (Brain et al., 1978; Zochi et al., 1994) have also been reported. Thus, it seems that susceptibility to the isolation and the cohabiting-induced aggression differs between strains. In our study we have used male mice of the OF1 outbred strain and the results show that this strain is susceptible to a display of aggression after both paradigms.

On the other hand, no significant differences were found in any of the other five behavioral categories recorded. Only a few studies have also paid attention to other behaviors displayed by the animals during the agonistic encounter. To this respect our results differ from those carried out on TO male mice in which males cohabitating with a female spent less time in social exploration and more time in non social exploration than isolated mice (Brain et al., 1978). As seen in the results, no differences were found between the two groups in our study in either of those behaviors.

Two important questions arise from these studies: 1) what are the mechanisms underlying the induction of aggression by these two paradigms? and 2) what are the genetic differences involved in the differences between strains? In addition, it is important to bear in mind that the changes produced by these two living conditions might influence the results of the pharmacological studies carried out to assess the biological bases of aggression.

In conclusion, this study shows that isolation and cohabitation with a female induce the

Table 2
Total duration, frecuency and latency (median with ranges) of each broad category of behavior by isolated or cohabiting with a female male mice over an agonistic encounter with an «anosmic» opponet

BEHAVIORAL CATEGORIES	TIME SPENT (seconds)		FREQUENCY		LATENCY (seconds)	
	Cohabiting with a female	Isolated	Cohabiting with a female	Isolated	Cohabiting with a female	Isolated
BODY CARE	9.3 2.4-23.6	10 4.4-25.9	8 13-17	10 2-29	92.8 368.9-397.4	119.4 50.4-404.9
DIGGING	0.7 0-53.4	3.5 0-12.9	2 0-27	2 0-14	487.2 144.2-600	455.3 212.3-600
NON-SOCIAL EXPLORATION	389.2 261.7-533.1	360.6 287.6-444.9	58 43-85	64 51-90	0.2 0.1-0.5	0.2 0.2-0.4
EXPLORATION FROM A DISTANCE	9.3 1.1-27	4 0-39.7	11.5 2-26	6 0-31	42.7 3.3-93.3	35.8 5.5-600
SOCIAL INVESTIGATION	123 44.8-234.3	132.2 32.1-292.6	43.5 27-60	47 23-68	15.3 3.1-52.3	6.9 1.9-24.7
THREAT	12.4 0-78.9	31.5 0-96.6	15 0-35	25 0-70	257.8 55.8-600	217.3 5.4-600
ATTACK	31.4 0-64.1	30.7 0-125.6	10.5 0-30	10 0-44	405.9 83.5-600	244.7 8.1-600

* No defense, avoidance/flee, immobility or sexual behaviors were recorded.

same level of aggression in OF1 male mice. Considering that cohabitation with a female is more natural than the isolation paradigm, we should think about the possibility of using the former paradigm in studies on intermale aggression in this outbred strain of mice.

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