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ETHOLOGICALLY-DETERMINED RESPONSES OF MALE MICE IN NEW DYADIC ENCOUNTERS REFLECT THEIR PREVIOUS 'SOCIAL STATUS'

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This study examines the importance of dominance or submissiveness in pairs of mice, on their performance in subsequent agonistic encounters. Animals were housed in pairs for three days, and, on the basis of their behaviour patterns (attack, threat, submission, avoidance), were classified as dominants or subordinates. Subsequently, behaviour of dominants confronting dominants, dominants confronting new subordinates and subordinates confronting subordinates were video-taped and behaviour evaluated using an ethologically-based analysis. Submissive and dominant animals showed behavioural characteristics that clearly reflect their previous social status and were less influenced by an immediate evaluation of the opponent. In the course of this experiment, dominant animals showed relatively little defensive/avoidance behaviour, irrespective of the type of opponent. Submissive counterparts, acutely showed no offensive behaviour when confronting dominants or submissives. Behavioural elements other than strictly agonistic ones are influenced by the previous status of male mice.

La conducta de ratones macho ante nuevas interacciones diádicas, evaluada etológicamente, refleja su 'estatus social' previo. El presente estudio examina la importancia de la dominancia o sumisión en parejas de ratones, sobre su conducta en posteriores interacciones agonísticas. Los animales fueron alojados en parejas durante tres días y, en función de sus pautas de conducta (ataque, amenaza, sumisión, evitación) fueron clasificados como dominantes o subordinados. Posteriormente, se filmó la conducta de ratones dominantes enfrentados con dominantes, dominantes enfrentados con subordinados y subordinados enfrentados con subordinados, y tal conducta fue evaluada utilizando un sistema de análisis de tipo etológico. Tanto los animales sumisos como los dominantes mostraron características conductuales que reflejan su estatus social previo, y su conducta se vio menos influída por una evaluación inmediata del oponente. En el curso de este experimento, los animales dominantes mostraron relativamente pocas pautas de conducta defensiva o de evitación, independientemente del tipo de oponente. Por su parte, los animales sumisos, no mostraron conductas ofensivas cuando se enfrentaron con dominantes ni cuando lo hicieron con sumisos. Además, el estatus previo de los animales influyó sobre elementos conductuales no estrictamente agonísticos.

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Numerous forms of behaviour have been described as 'aggression', varying in terms of the situations in which they occur, their presumed functions and underlaying phy-

siologies (Moyer, 1968, Brain, 1981). Because attack and defensive/submissive behaviours are intimately related, many scientists prefer to use the term "agonistic behaviour" to encompass the entire continuum involved in conflict. The agonistic interaction that has been most studied is that of intraspecific aggression between males. Such behaviour is 'ritualized', in that it seems to involve injury-limiting strategies, and its function seems to be the increasing of the possibility of gaining access to a resource (a mate, territory or social status), closely concerned with reproduction.

The concept of dominance where one animal takes precedence over another is intimately associated with the above type of aggression. Much controversy surrounds the dominance-subordination relationship, although a consensus holds that it is a relationship rather than a characteristic and it bestows the benefits of fighting without the repeated costs (Richards, 1974; Syme, 1974; Bernstein, 1980, 1981; Benton and Dalrymple-Alford, 1981). The asymmetries of agonistic interactions are not only due to the physical characteristics of participants and to their innate aggressiveness; but are also influenced by their general and specific previous experiences, and the type of opponent (Martínez, Salvador and Simón, 1994). Experience of social aggression leading to a victory clearly augments attack in subsequent encounters of the same kind in rats and mice, whereas experience of defeat has the opposite effect (e.g. Lagerspetz, 1964; Brain and Poole, 1974; Goldsmith, Brain and Benton, 1976; Ely and Henry, 1978; Van de Poll, Van Oyen and Van Pelt, 1982). Indeed, mice given repeated opportunities to fight show more attack than individuals lacking such experience (Parmigiani and Brain, 1983).

The present study attempted to analyse the impact of previous social status on performance in subsequent agonistic encounters, a factor likely to be of especial importance in determining the outcome of agonistic interactions. Experimental opponents were selected for the study with varied previous social status. An attempt was essentially made to determine whether prior social status influenced agonistic behaviour per se (threat, attack, defense/submission, avoidance/fleeing) as well as other elements of mouse behaviour (e.g. body care, non-social exploration and digging) shown in dyadic encounters.

Materials and Methods

Subjects and husbandry

Seventy eight, 42 day old albino OF1 strain mice from IFFA Credo were used in this study which was carried out in the Animal Facilities of the Faculty of Psychology (San Sebastián). The animals were individually housed for 4 weeks in transparent plastic cages measuring 24.5 x 24.5 x 15 cm (Panlab, Barcelona). The holding room was maintained at a constant temperature of 21 °C and an artificial light/dark cycle (white lights on 01:00 to 13:00 hr local time) was in operation. Food (Panlab, Barcelona) and water were available ad libitum.

General procedure and behavioural tests

At the end of isolation period, subjects were randomly allocated to pairs. Each pair of animals was observed for ten minutes on each of three consecutive days. Those animals that showed submissive behaviour patterns and subsequently never showed attack or threat patterns, were considered submissives. Counterparts that never showed submission patterns but evidenced attack and threat, whilst their cagemates showed submission, were considered dominants. In this way, a dominant and a submissive animal was distinguished in each cage. Five pairs

that did not fit these criteria were eliminated from the study. Subsequently, three main confrontation categories were staged between unfamiliar pairs of subjects, namely:

(a) Dominant (n=12) vs new subordinate (n=12),
(b) Dominant (n=11) vs dominant (n=11),
(c) Subordinate (n=11) vs subordinate (n=11).

The encounter consisted of 10 min of free interaction between pairs of subjects (confrontation categories) in a neutral cage measuring 50 x 50 x 25 cm. The interactions were video-taped (Blaupunkt RTX-260; normal light conditions) and the encounters were evaluated using a program covering 51 behavioural elements grouped into 11 broad functional categories, namely: body care, digging, non social exploration, exploration from a distance, social investigation, threat, attack, avoidance/flee, defensive/submissive, sexual and immobility. Only the accumulated times allocated by animals were assessed. The categories, their constituent elements and the program are described in Brain, McAllister and Walmsley (1989) and Martínez, Castaño, Simón and Brain (1986).

Statistical analysis

The data were initially analysed using the Kruskal-Wallis test (Siegel, 1956). Appropriate post hoc comparisons were then made using two tailed Mann-Whitney U-tests. Following comparisons were made:

(i) dominant subjects confronting a new subordinate.
(ii) subordinate subjects confronting a new dominant.
(iii) dominant subjects confronting another dominant.
(iv) subordinate subjects confronting another subordinate.

Results

The median times with ranges allocated to broad categories of behaviour in social interactions for the four types of interaction are given in Table 1. No significant levels of sexual behaviour were observed in any category, so this item is not included in the table. Kruskal-Wallis tests (also see Table 1) revealed that digging, non social exploration, social investigation, threat, attack, avoidance/fleeing and defensive-submissive behaviour categories showed significant variance across the four interaction types. Additionally, Mann-Whitney comparison-tests between categories showed the following significative differences.

Predictably, the dominant animals confronting subordinates showed significantly more digging ($U = 1$, $P < 0.002$), non social exploration ($U = 12$, $P < 0.002$) , threat ($U = 0$, $P < 0.001$) and attack ($U = 0$, $P < 0.001$), whereas the subordinate animals paired with other subordinates, showed significantly more avoidance/fleeing ($U = 25$, $P < 0.02$) and defensive/submissive ($U = 0$, $P < 0.002$) behaviours. Social investigation did not significantly differ between the groups.

Dominants confronting subordinates showed significantly more social investigation ($U = 24$, $P < 0.02$) and non social exploration ($U = 26$, $P < 0.02$) but less defensive/submissive behaviour ($U = 30$, $P < 0.05$) than dominants confronting other dominants. Digging, threat, attack and avoidance/fleeing were not significantly different in these groups.

Subordinates confronting dominants showed significantly more avoidance/fleeing ($U = 5$, $P < 0.002$) and defense/submission ($U = 0$, $P < 0.001$) but less digging ($U = 6.5$, $P < 0.002$) and social investigation ($U = 10$,

Table 1
Medians (with ranges) for allocated to broad behavioural categories in social interactions of each pairing group

Focus animal / Oponent / Behavioural categories	Dominants (n=12)	Subordinates (n=12)	Dominants (n=11)	Subordinates (n=11)	Kruskal-Wallis value plus associated probability
	Subordinates	Dominants	Dominants	Subordinates	
Body Care	64.64 (022.05-120.41)	41.63 (002.35-108.08)	159.85 (005.94-193.78)	71.05 (034.19-209.30)	h=6.226 n.s.
Digging	37.74 (005.44-128.54)	0.00 (000.00-010.66)	11.84 (000.00-064.50)	5.79 (000.20-062.32)	h=23.467 P< 0.001
Non Social Exploration	271.06 (185.73-362.52)	200.09 (072.44-251.44)	194.64 (129.76-292.00)	231.83 (011.23-372.65)	h=10.895 P< 0.01
Exploration From a Distance	22.60 (010.29-052.83)	40.02 (017.57-096.32)	36.08 (013.94-135.20)	34.22 (006.08-082.03)	h=3.931 n.s.
Social Investigation	50.12 (008.24-122.95)	36.74 (002.64-074.29)	5.68 (000.00-136.87)	134.92 (021.89-173.91)	h=19.358 P< 0.001
Threat	22.50 (010.76-109.76)	0.00 (000.00-000.00)	50.52 (001.89-106.41)	0.00 (000.00-058.65)	h=28.647 P< 0.001
Attack	51.37 (010.38-147.57)	0.00 (000.00-000.00)	57.21 (019.70-136.84)	0.00 (000.00-000.88)	h=36.789 P< 0.001
Avoidance Fleeing	7.74 (000.00-075.25)	38.64 (007.17-071.75)	8.48 (003.63-148.19)	6.25 (000.00-025.59)	h=14.755 P< 0.001
Defense Submission	0.00 (000.00-000.00)	212.39 (143.77-402.19)	0.22 (000.00-172.05)	8.95 (000.00-064.51)	h=32.28 P< 0.001
Immobility	2.41 (000.00-045.32)	15.93 (000.00-103.50)	2.72 (000.00-132.05)	6.27 (000.00-216.00)	h=6.534 n.s.

$P < 0.002$) than subordinates confronting subordinates. There were no significant differences in non social exploration, threat and attack behaviours between these two groups.

Dominants confronting dominants showed significantly more threat ($U = 11$, $P < 0.002$) and attack ($U = 0$, $P < 0.001$) but less social investigation ($U = 9$, $P < 0.002$) than subordinates confronting dominants. There were no significant differences in digging, non social exploration, avoidance/fleeing and defense/submission parameters.

Discussion

The impact of social status on behaviour in the different encounters will be considered behaviour by behaviour.

Agonistic behaviour

The fact that dominant animals confronting unfamiliar submissive opponents showed more offensive and less avoidance behaviour than subordinate animals confronting dominant counterparts is not especially

remarkable. The fact that animals in this latter group showed *no* offensive behaviour is interesting, as is the fact that defensive/avoidance behaviour was rare in dominants confronting new subordinates. These findings agree with those obtained by Blanchard and Blanchard (1986) who confronted resident (dominant) with intruder (subordinate) rats. The residents showed only offensive behaviours, while the intruders showed mainly defensive behaviour. In the same way, Kamal (1986) showed that male Swiss albino mice with positive fighting experience evidence more threat and attack behaviour towards intruders than did counterparts lacking such experience. Blanchard and Blanchard (1981) suggest that experience with intruders increases aggressive behaviour, reducing fear of intruders by increasing familiarity with this class of stimuli. In the present study whether dominant subjects confronted submissive or dominant partners had no significant effect on their levels of offense. However, an increase in defensive/submissive behaviour was evident in those dominant subjects encountering other dominants, a clear consequence of the generated fighting. Dominants encountering submissives lacked an aggressive opponent to elicit much defensive/submissive behaviours. Clearly, the type of opponent encountered has a major influence on the defensive behaviour shown by dominants.

On the other hand, submissives encountering dominants or other submissives showed no offensive behaviour. Submissives encountering dominants showed more defensive and avoidance behaviour, as they were confronted with aggressive subjects. The fact that submissives encountering submissives showed no attack reflects the fact that mice with previous defeat experience need a minimum of three days to recover to a base line of offensive behaviour, if they have not had previous positive fighting experiences (Andrade, Kamal and Brain, 1989).

Non agonistic behaviour

Subjects that had prior dominance experience explored vigorously and showed much digging when confronting submissive partners. Submissive animals in this context showed inhibition of exploration. In the same way, when submissive subjects confronted submissive partners they showed more digging than counterparts confronting dominants.

It would be interesting, in future, to study the possible significance of the digging behaviour in that it may be related to marking and maintaining the territory. Perhaps, some of the behavioural patterns that do not have a strictly agonistic connotation are susceptible to modifications, according to the opponent type as well as previous experience. These patterns appear to have a greater functional flexibility than specifically agonistic behaviour as suggested by Fernández-Espejo and Mir (1990).

It seems clear that submissive and dominant animals in the present context show behavioural characteristics that reflect their previous social status and these are less influenced by an immediate evaluation of the opponent. The nature of the opponent does, however, modify behaviour in a predictable manner. This study also shows that elements other than those strictly concerned with agonistic behaviour are influenced by previous and immediate experiences. The data at first sight consequently run counter to the initially reported consensus that dominance is part of a *relationship* rather than an individual *characteristic.* Clearly we could argue that, at least acutely, dominant mice tend to behave as dominants in a new situation and subordinates as subordinates. There is, however, another interpretation. Laboratory mice are derived from ancestors in which the male is frequently highly territorial (Brain and Parmigiani, 1990; Brain, 1992). Perhaps 'dominants' and 'subordinates' here are sub-

jects with respectively a high and a low propensity for showing territoriality in a novel arena. The detailed ethological analysis (especially the non-agonistic elements) provides some support for this view in that a non-threatening partner elicited more digging and social investigation. Digging and exploration seem to be aspects of territoriality.

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