The mice used in animal facilities belong to the family Muridae, subfamily Murinae, order Rodentia, genus Mus and species Mus musculus. They are social and territorial animals. Several lineages developed in the laboratory show aggressive behavior similar to that of wild mice. The aim of this study was to characterize the hierarchical structure, body characteristics and hormonal levels in dominant, subordinate and non-aggressive mice. The model established for spontaneous aggression (MSA) evaluates the pattern of aggressive behavior (PBA) and categorizes the animals as follows: LAg - individuals with low aggressive behavior, HAg - dominant (highly aggressive) and Sb - subordinate. The regrouping of adult male mice produces substantial stress and influences the formation of their hierarchical structure. Behavioral comparisons before regrouping (BfR) and after regrouping showed that the percentage of body weight lost in mice was dependent on the specific categories as follows: LAg - 68.1%, HAg - 86.9% and Sb - 90.5%; the average corticosterone levels by category were BfR: 43.5±17.5, LAg: 177.0±40.4, HAg: 72.8±23.8 and Sb: 136.4±51.2 ng/mL. Dominant mice showed differences in body characteristics (primarily the body/tail relationship) relative to subordinate mice. Two additional hierarchical positions were observed: “neutral individual” (without aggressive behavior) and “subordinate target” (an animal that receives approximately 30% of the total number of attacks made by dominant animals).

group structured by competition (with or without violence). This dominance hierarchy may also be characterized as linear or despotic. The linear form is characterized as a relationship between individuals without centralized dominance, whereas the despotic form includes only one dominant individual, with the others equally submissive.

The factors that determine dominant and subordinate status are not entirely known. It has been suggested that the animal’s behavior, physiological or health status, fighting/defensive ability and previous experience are important for the hierarchical structure. Hilakivi et al. (2009) observed behavioral, hormonal and neurochemical differences between submissive and dominant mice; the submissive mice showed elevated 5-HIAA levels in the hypothalamus, hippocampus and brainstem. In contrast, the dominant mice had decreased concentrations of dopamine in the brainstem. The steroids cortisol and testosterone have become well-established targets in the search for hormonal modulators of social aggression. Testosterone activates the subcortical areas of the brain to produce aggression, whereas cortisol and serotonin act antagonistically with testosterone to reduce its effects. Previous research has shown that alterations in the hypothalamic-pituitary-adrenal (HPA) axis are correlated with antisocial and aggressive behavior. Some evidence suggests that low cortisol levels may function as a biological marker for a severe antisocial subgroup with pronounced callous-unemotional (CU) traits and appear to be more closely related to a general deficit in behavioral regulation.

Our working group has implemented a model for behavior evaluation to identify dominance, aggression and subordination induced by the grouping/regrouping of the same animals and based on monitoring from youth to adulthood (MSA). We have observed that adult Swiss Webster male mice form a dominance hierarchy, with high-intensity (despotic) aggression in approximately 70% of the grouped animals. The aim of the present study was to determine the characterization of hierarchical structure, body morphometry and corticosteroid differences among dominant (aggressive), subordinate (attacked) and harmonious (non-aggressive) Swiss Webster mice after regrouping.

### 2 MATERIAL AND METHODS

#### Animals

Male albino Swiss mice (3 weeks old) were maintained in our animal facilities at the Division of Animal Experimentation of the Cell Biology Laboratory, Instituto Oswaldo Cruz (SEA/LBC-IOC). They were adapted to the environment for 1 week in ventilated racks, and the temperature, humidity and photoperiod were controlled according to the standard environmental regulations. The animals were maintained under stable conditions of temperature and light with a 12-h light/dark cycle, and both food and water were available ad libitum. Routine cleaning was performed twice per week. The procedures were performed under license number LW-5/12 of the Ethics Committee for the Use of Animals (CEUA/FIOCRUZ).

#### Model of spontaneous aggressiveness (MSA)

The mice were distributed into 5 groups (A1 to A5) of 10 mice each, and the mice in each group were individually identified (c1 to c10) (Scheme 1). Three behavioral assessments (described below) were used for all animals once per week in the 4th, 6th and 8th weeks of life. During the 10th week, the animals were regrouped. A raffle procedure was used to assign individuals to new groups (designated R1 to R10) of 5 mice each (with individuals designated c1 to c5). Regrouping occurred without any interference or personal choice by the experimenter. Behavioral assessments were performed once per week in the 12th, 14th and 16th weeks of life. Two grouping categories were used: before regrouping (BfR) and after regrouping. The following behavioral categories were defined: LAg – no aggressive
events, HAg - highly aggressive mice (dominant) and Sb – mice suffering attacks (subordinates).

**Behavioral Analysis**

**Ethogram**

We recorded (top view) each group (4th, 6th, 8th, 12th, 14th and 16th weeks) for 60 continuous minutes using a Canon PowerShot SX20 IS® video camera (Canon, Lake Success, New York, USA). A total of 3600 minutes of video were recorded, and the most representative changes were further documented with photography. From these video records, we determined the variables to be evaluated in the ethogram: a) the pattern of aggressive behavior (PBA), including bites, wounds and injuries to an animal caused by fights among individuals in each group, and b) the qualitative and quantitative PBA intensities, determined by a scoring system. The following scoring system was used: 0 (zero): the absence and/or presence of vocalizations and persecution, with no signs of bites or lesions on the animal’s body; 1+: the occurrence of a small number of aggressive events, with or without sexual characteristics (attempted intercourse between individuals) and with small bites or injuries occurring anywhere on the body; 2+: the occurrence of a small number of aggressive events without bites with sexual characteristics and discrete marks on the tail, back or scrotum; 3+: the occurrence of a moderate number of aggressive events and the observation of injuries and mild lesions on the tail, back and scrotum of the animals; and 4+: a high frequency (or intensity) of aggressive events and the presence of marked lesions and injuries on the tail, back and scrotum. In certain cases, injuries of varying intensities were observed on other parts of the body, such as the chest, abdomen and forelegs (defensive injuries).

**Evaluation of body weight**

In all groups, the body weight and weekly weight gain of all individuals were monitored throughout the experiment. Our evaluation was based on the following criteria: a) a comparison between the average body weight (grams) of the individuals before regrouping (BIR at the 10th week of life) and the respective categories defined as follows (by PBA levels) after regrouping: LAg – no aggressive events, HAg – highly aggressive and Sb – subordinates at 16th weeks of life; b)

![Figure 1: Structure and development of the MSA: 3 week old male Swiss Webster mice were separated into 5 groups (A1 to A5) with 10 animals per group. During the 4th, 6th and 8th weeks, each animal was monitored with an ethological analysis (ethogram), body weight measurements and morphometric evaluations. In the 10th week, the animals were randomly regrouped in 10 new cages (R1 to R10), and the behavioral tests were repeated for all animals in the 12th, 14th and 16th weeks.](image)
a comparison between the average weight gain (grams) per week among individuals before regrouping (BfR – between the 4th and 8th weeks of life) and the categories LAg, HAg and Sb between the 12th and 16th weeks of life.

Morphometric analysis

After the 16th week of life, the animals were euthanized and the following measurements (mm) made with a digital pachymeter (Digimess – China): a – total length (tip of the nose to the tip of the tail); b – head length (occipital area of skull to the tip of the nose); c – body length (thorax and abdomen); d – tail length; e – width of the head (between the ears); f – width of the abdomen; and g – width of the tail base.

Corticosterone level

Blood was collected in glass tubes and centrifuged at 3018.4 g for 15 min at room temperature to obtain serum and at 4°C to obtain plasma. Plasma corticosterone levels were assayed with a double-antibody radioimmunoassay method specific for rats and mice using a commercial kit (MP Biomedicals, USA). The sensitivity of the assay was 0.25 ng/mL.

Statistical analysis

A Mann-Whitney non-parametric test was used to perform between-group comparisons (SPSS software, version 8.0). The p values are shown in the figure legends.

The ethogram profile showed differences in the PBA levels (Fig. 3). Certain cages showed high intensities of aggression (HAg), with 55 attacks/60 min and the individual PBA (PBAInd) values equal to 0 for the dominant animal and from 1+ to 4+ for the subordinate animals. We also observed cages with an average level of aggressiveness (Med) of 24 attacks/60 min, with PBAInd ranging from 0 (dominant) to 3+ (subordinate), and cages with low aggressiveness (Low), with PBAInd between 0 and 1+ (Fig. 3A). At each of the three PBA levels, we also observed other individuals with PBAInd = 0. We applied the term “neutral individuals” to individuals that did not attack others and were rarely attacked by the dominant. In contrast, we observed subordinate individuals that received 20-30% of the total attacks by the dominant. These individuals, termed “subordinate targets”, showed PBAInd values between 3+ and 4+ (Fig. 3B).

The morphometric analysis showed that the total length in dominant mice (HAg) (212.6±4.6
mm) was greater than the total length in mice belonging to the other categories (LAG: 211.5±2.8 mm; Sb: 206.3±3.2 mm) (Table 1, Fig. 4). This size difference was primarily due to the length of the tail (HAg: 102.9±1.9, Sb: 98.2±2.6, and LAg: 99.9±0.8 mm), as the body lengths were similar. Furthermore, the abdominal width of the dominant mice (HAg: 39.7±2.1 mm) was less than that of the LAg mice (43.8±1.1 mm). The body length (106.±3.0 mm) and abdominal width (35.0±1.7 mm) of the Sb mice (subordinates) were less than those of the LAg mice: 111.6±6 mm and 43.8±1.1 mm, respectively. Finally, we emphasize that the tail length of the HAg mice (4.8±0.2 mm) was significantly higher than that of the mice belonging to the other categories. Additionally, the tail width of the HAg mice was greater than that of the mice belonging to the other categories. Generally, the mice receiving attacks (subordinate mice) were smaller and thinner and their tails smaller and thinner in comparison to the corresponding values for the other categories.

Significant differences in body weight were also observed (Fig. 5). The LAg group showed a body weight value (49.3±1.9 g) that was greater
than the body weights for BfR (43.1±4.2 g), HAg (45.6±2.5 g) and Sb (43.8±1.8 g) (Fig. 5A). Another important finding was that the weight gain per week decreased in all categories. Between the 4th and 8th weeks of life, BfR showed an average weight gain of 19.7±3.6 g. The regrouping of the mice produced a significant decrease in weight gain. Between the 12th and 16th weeks of life, the LAg group showed a lower value of weight gain (6.1±2.4 g), as did the HAg (2.5±0.5 g) and Sb (1.8±0.3 g) groups (Fig. 5B). The weight gain after regrouping, expressed as a percentage of the BfR weight gain, showed a marked decrease: LAg - 68.1%, HAg – 86.9% and Sb - 90.5% (Fig. 5B).

Significant differences were observed in the plasma levels of corticosterone in all categories at the 16th week (Fig. 6). Before regrouping, the average value was 43.5±17.5 ng/mL. The regrouping may be considered a stressful stimulus that induced higher levels of corticosterone. The LAg group increased to 177.0±40.4 ng/mL, the Sb group to 136.4±51.2 ng/mL. However, HAg individuals did not show a proportional increase, maintaining a level of approximately 72.8±23.8 ng/mL. Despite the large variation shown by the individual corticosterone values, we found that the BfR value differed significantly (p ≤ 0.001) from all the other categories. Moreover, a significant difference was observed between LAg and HAg (p ≤ 0.001) but not between LAg and Sb (p ≥ 0.125).

Table 1: Morphological body measurements.

<table>
<thead>
<tr>
<th></th>
<th>Total Length (mm)</th>
<th>Head Width (mm)</th>
<th>Tail Head Abdomen Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>BfR</td>
<td>203.6±3.9</td>
<td>27.8±0.7</td>
<td>107.4±2.8</td>
</tr>
<tr>
<td>LAg</td>
<td>211.5±2.8</td>
<td>28.9±0.3</td>
<td>111.6±2.2</td>
</tr>
<tr>
<td>HAg</td>
<td>212.6±4.6*</td>
<td>28.7±1.0</td>
<td>107.7±2.5</td>
</tr>
<tr>
<td>Sb</td>
<td>206.3±3.2</td>
<td>28.8±0.4</td>
<td>106.8±3.0*</td>
</tr>
</tbody>
</table>

Values are means ± SD  # Sb vs. LAg (p<0.05)  @ HAg vs. LAg (p<0.05)  * LAg vs. Sb (p<0.05).
4 DISCUSSION

Among Crowcroft’s contributions is his important book, *Mice All Over*, which describes pest control research in the 50s. This author investigated mouse populations in a near-natural setting; his experiments revealed the complexity of their society. Observations of trapped wild mice found that aggressive territoriality occurred. Each territory was surrounded by an invisible line of demarcation beyond which an intruder would elicit attack by the resident male. These resident males were relegated to the bottom of the social hierarchy, were attacked when encountered by other males and shared a home box, possibly for protection against aggressive marauders.

Crowcroft (1996) also compared the behavior of wild and lab mice. Initially, lab animals were
disinterested and unaggressive, suggesting that
certain typical behaviors were suppressed by cage-
rearing. However, their original behavior, i.e.,
chasing and fighting similar to that of wild mice,
subsequently returned, and a social hierarchy was
formed\(^{19}\). If a wild mouse then happened to inva-
de the territories of these lab mice, the incomer
was overpowered by the residents and dropped
to the bottom of the hierarchy\(^{19,20}\). Our results
demonstrate that male mice of the Swiss Webster
lineage maintained in a laboratory environment
show behavior consistent with Crowcroft’s ob-
servations, with emphasis on social hierarchy,
aggression and territoriality.

However, unlike the mice in Crowcroft’s study,
our animals did not have a choice of boxes or an
opportunity to escape. We believe that the restric-
tion of an animal to a limited area can facilitate
aggression. Interestingly, we observed different
intensities of aggression in terms of the number of
attacks and the PBA degree. In a similar manner,
the Swiss Webster social hierarchy, traditionally
associated with competition for limited environ-
mental resources and social dominance\(^{21}\), was also
related to hyperaggressiveness and the “tyrannic”
hierarchy observed in bank voles (Clethrionomys
glareolus) housed in a restricted cage milieu\(^{22}\).
Moreover, factors intrinsic to the individual (rela-
tive to an aggressive context), such as genetics\(^{20}\),
neurological or emotional trauma in intrauterine
and postnatal life\(^{23,24}\), may directly influence the
aggressiveness of the dominant animal.

In standard methodologies used to study animal
conflict, a pair of animals is used, and the only
possible outcomes are the identification of the
aggressor and the animal attacked or the definition
of dominant/submissive roles in a hierarchy at
a given time of observation (generally 5 minu-
tes)\(^{2,10,25,26}\) Our MSA technical application offers
the following three advantages: (1) monitoring of
the same animal during weaning and childhood;
(2) identifying the individuals most likely to
become aggressive; and (3) observing group
formation, interactions between individuals and
hierarchical structure\(^{2,10}\). Initially, a clear defini-
tion of hierarchy, dominance and injuries resulting
from attacks was not obtained for individuals in
the group (4\(^{th}\) to 8\(^{th}\) weeks of life). During the
regrouping (adulthood until the 16\(^{th}\) week of life),
however, it was possible to observe the classical
social categories (dominant/subordinate) and also
a “neutral” category, whose members, for some
reason, suffered no injuries and was not attacked
by other animals. In the subordinate category, our
observations emphasize the presence of an indi-
vidual who suffered approximately 20-30% of the
attacks by the dominant animal. This individual
was called the “subordinate target”.

To develop a better understanding of the hie-
rarchy and of social status in the experimental
animals, we compared individuals in highly ag-
gressive groupings with individuals showing a low
incidence of aggression and evaluated morphome-
tric measurements collected on the following cate-
gories: mice before regrouping (BfR), individuals
with low levels of aggressive behavior after regrou-
ping (LAg), animals performing attacks, dominant
animals (HAg) and subordinates (Sb). In nonhu-
man primates, aggressive behavior links the search
for resources with body size and physical power,
factors that determine the highest dominance rank
through disputes in the group hierarchy\(^{27,28,29,30}\).
In Swiss Webster mice, our results demonstrated
that body weight and/or muscular strength are not
significantly related to dominance/subordination
(data not shown). Given the evolutionary potential
for specialized functions in organs and limbs, we
hypothesize that the tail of the dominant individual
functions to achieve optimal balance for the body
and to provide strength to intensify the dominant
animal’s aggressive attitudes\(^{31}\).

The body weight and weight gain data obtained
in this study imply that the regrouping of mice
in adulthood induced a stress situation in all the
categories of individuals examined. This results
is consistent with the literature on this topic\(^{32,33}\).
Furthermore, we observed that the animals be-
longing to the HAg group and the subordinates
were more affected by stress. However, the HAg
corticosterone levels were not directly related to
these acute stress states. After regrouping, the
hormonal levels of the HAg animals were lower
than those of the animals in the other categories. This finding is similar to previous results for patients with posttraumatic stress disorder (PTSD)\textsuperscript{16}. Neurobiological findings have shown that PTSD is associated with hypothalamic-pituitary-adrenal (HPA) axis dysfunctions and with other brain structures such as the prefrontal cortex, hippocampus and amygdala. These patients have low plasma levels of cortisol and present increased glucocorticoid receptor responsiveness, suggesting that the inhibition of negative feedback plays a significant role in the pathology of the disorder\textsuperscript{16}.

Moreover, the tails of the dominant animals were wider than those of the animals in the other categories. We consider that high levels of aggression are associated with low basal levels of corticosterone, hypothalamic-pituitary-adrenal (HPA) axis dysfunctions and other brain structures, suggesting the occurrence of PTSD before regrouping in the experimentally controlled environment.

We thank Dr. Solange Lisboa de Castro for a critical review of the manuscript and Dr. Maria de Nazaré Corrêa Soeiro, head of the Laboratório de Biologia Celular (IOC/Fiocruz), for logistical support. We also acknowledge financial support from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (#11/12325-6 and #12/05396-7) and Conselho Nacional de Desenvolvimento Tecnológico e Pesquisa (CNPq).
Resumo

Comparações comportamentais antes (BfR) e após reagrupamento mostrou que o percentual de peso perdido por animais nas categorias especificadas foram as seguintes: Lag - 68,1%, HAg - 86,9% e Sb - 90,5%; os níveis médios de corticosterona por categoria foram BfR: 43,5 ± 17,5, LAg: 177,0 ± 40,4, Hag: 72,8±23,8 e Sb: 136,4±51,2 ng/mL. Camundongos dominantes apresentaram diferenças nas características do corpo (principalmente a relação corpo/cauda) em relação aos indivíduos subordinados. Também foram observadas duas posições hierárquicas adicionais: "indivíduo neutro" (sem comportamento agressivo) e "subordinado alvo".


Referências

22. Sorensen G. Stereotyped behaviour, hyperaggressiveness


