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Research Article

Influence of social isolation and aggressive behavior in the appearance of Depression-like in Experimental model

Abstract

The study of animal behavior in the laboratory environment aims to promote welfare and minimize the discomfort of animals during scientific tests. This knowledge is important to understand social interactions and develop methods of environmental enrichment, thus increasing the quality of life of animals housed in the laboratory. The goal this study was evaluated the individual and social behavior and influence in the anxiety or depression-like condition in mouse during aggressive behavior and compared with social isolation in the animal facilities. Observing their daily routine, the presence of aggressive behavior patterns of individual mice, mainly male adult mouse. We used two ethological methods: i) Open-field and ii) Elevated plus-maze test in the three categories of behavior observed, dominant, subordinate and isolated mice. Our results showed that in groups where there was the presence of aggressive behavior patterns (in both ethological tests) motor and exploratory activity in dominant and subordinate categories was inferior when compared with to harmonic interaction group. Furthermore, subordinate and isolated mice showed a significant decrease in activity, compatible with depressive behavior described in the literature for experimental models. Concluded, from these results we were able to observe that social disturbance and aggressive behavior during social and individual interaction promoted discomfort and stress in mice lab and depression-like state.

Introduction

Actually, there is intense discussion related to the laboratory animal welfare in biomedical testing. Ethical parameters in the use of laboratory animals are, mainly, based in the 3Rs principles [1]. Briefly, these principles suggest: i) substitution of animals by alternative methods; ii) reduction in the number of animals used and iii) refinement of housing and manipulation techniques. In non-human primate model various studies have addressed the question of promoting an environment for individuals to develop their exploratory activity and increase the quality of social interaction [2]. Accordingly, studies recommend trying to find better techniques for the maintenance of laboratory animals to promote the welfare of individuals and minimize animal discomfort [2,3]. For rodents there are also several studies related to environmental enrichment [4,5]. However, indexed literature analysis described (Pub Med - August 2019) a relatively small amount of research into the individual and social behavior of mice. About 49,763 studies have evaluated the issue of welfare in non-human primates, whereas only 316 corresponding works related on mice [6]. Hence, the perceived necessity for the study of mice behavior in the laboratory in order to promote knowledge that could be used in the development of new equipment, procedures and techniques, improving the welfare of laboratory mice. Furthermore, we observe that this knowledge in the animal behavior is also important to improve the empathy of the manipulator in relation to the animal, increasing the quality in the procedures performed and minimizing the occurrence of biological accidents [6].

Territorialim, sexual selection and social interaction was intrinsic characteristic of the mouse lab and dominant behavior can be observed in both male and female mice. In the adult male mice, the aggressive behavior, there is high prevalence of behavior between adult individuals. The appearance of these fights is easily observable during routine cleaning and handling of these animals [7]. The aggression between male mice may have several causes: genetic [8], hormonal [9,10] or neurochemical [11,12] among others. It´s suggested that this aggression could be related to the struggle of individuals for social domination within the structural hierarchy [13]. However, some studies suggest that the presence of aggression occurs because of the failure of the harmonic hierarchy [14]. The

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presence of dominant and subordinate (attacked) individuals was observed in several male groups. We can identify mice that promote aggression and others who suffer attacks [15]. It is likely that mice suffering attacks are those that are in an inferior position in social dominance [16]. The social isolation of the aggressor animal is the main attitude in the management of aggressive animals [16].

The aim this study is to investigated the influence of the aggressive behavior in emotional state of the mice lab during housing in the animal facilities. We use motor and exploratory activity and pattern of aggressive behavior (PAB) in the all groups (and categories), and social isolation animal. Using ethological methods, normally employed for the evaluation of animal models of depression and anxiety [16], we measured the level of motor and exploratory activity in dominant, subordinate and isolated mice [17]. Then our proposal was to compare among the categories studied the presence of the state of anxiety and depression, mainly in the management of social isolation and in a situation of exacerbated aggression in groups of mice lab. Animals maintained in the absence of social interaction, described in the literature as a model for hypoactivity or depression-like behavior, were used as a reference [18,19].

Material and Methods

Mice

Male Swiss Webster mice were obtained from Instituto de Ciência e Tecnologia em Biomodelos (ICTB/Fiocruz) and maintained at LAboratory Animal Science Division of the Biotério de Experimentação Animal (LBC-LITEB – Instituto Oswaldo Cruz). The animals were adapted to the environment for one 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. The animals were male, adult Swiss Webster mice (over ten weeks of life). This project has license number (L – 10/18) at the Animal Ethical Use CEUA - IOC/FIOCRUZ. We used a total number of 3 to 4 animals per triplicate assay.

Pattern of aggressive behavior (PAB)

We evaluated the daily routine, looking for the appearance of signs of aggression, fights, bites and dominance in each animal group. We also noted other stress signs such as vocalizations, weight loss and others. Thus identifying and categorizing the groups and individuals who showed patterns of aggressive behavior.

Animal behavior categories: We divided the animals into four categories: i) Normal (norm), where none of the individuals had PAB in group. ii) Dominant (dnte), characterized by individuals in groups with PBA presence. iii) Subordinate (subt), related to animals that had bites and injuries in groups with PAB. iv) Solitary isolation (solit), mice that were housed without social interaction.

Open field test

This test consisted of the measurement of behavioral variables in experimental individuals, placed in an arena limited to sixteen spaces of equal size [20]. Behavioral assessment was carried out by evaluating motor activity by measuring the number of spaces crossed (horizontal displacements) by each mouse in a period of five minutes, while the exploratory activity was measured through the number of vertical displacements (rearing) independent of the location in the arena, also in a period of five minutes.

Elevated plus maze test

This test was used to assess the motor ability and exploratory interest of each animal [21]. The assessment of exploratory interest and locomotor ability was carried out through the measurement of the entry into and time spent by the individual mice in the closed and open arms over a period of five minutes.

Statistical analysis

Results were expressed as mean-standard deviation (±SD). Significance between means was determined using the Student t-test and results were regarded as significant when $P \leq 0.05$.

Results

Mice lab observation and PAB and isolation social behavior in their daily routine we divided them into respective categories. The behavior types was demonstrated graphically (Figure 1). The first category described is of mice that did not demonstrate aggressive behavior in the group, denominated as normal (Figure 1A). In relation to PAB groups we distinguished two categories of individual aggressive behavior. Dominant animals were those who showed no signs of fights or bites.

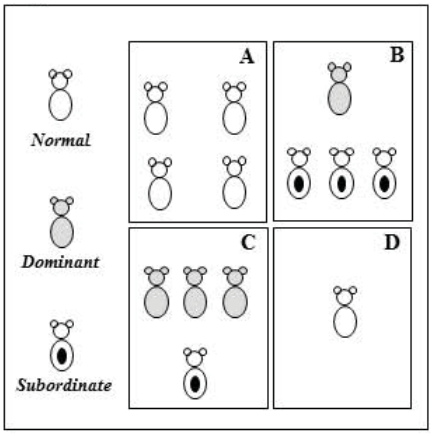

Figure 1: Ilustration of male mice behavior in laboratory environment: Our daily routine evaluation allowed the observation of normal mice with no alteration of behavior (white mouse), dominant mice (gray mouse) and subordinate mice (mouse with black spot). From this, we carried out the division of the categories into groups. Mouse group without presence of aggressive behavior (A). Animal groups with the presence of PAB were divided into two categories: "one against all" (B) where there is a dominant animal and "all against one" (C) where there are more dominant animals. We also evaluated the activity of animals housed in isolation from social interaction (D).

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Subordinate animals, in turn, showed signs of fights and bites. In these groups, two types of social dominance was suggested: i) «one against all» when an individual fought all components (Figure 1B) and ii) «all against one» when several components fought only one individual component of the group (Figure C), but we have not yet confirmed this dynamic of aggressiveness. Moreover, we also evaluated the activity of mice in social isolation (Figure 1D).

Open field test results (Figure 2) showed that both, dominant and subordinate mice, showed a significant decrease in motor (Figure 2A) and exploratory activity (Figure 2B) when compared to normal mice. Subordinate animals demonstrated lower activity when compared with the dominant mice and a similar level in relation to the animals in social isolation (Figure 2A,B) both in terms of horizontal and vertical displacement. Individual physical activity of the dominant and subordinated was evaluated of the two concomitants groups (Figure 3). The results demonstrated that each mice showed individual value of motor and exploratory activity. Interestingly, we observed a decreasing level in the individual profile of horizontal (Figure 3A-C) and vertical (Figure 3B-D) displacements of the mice in both cages, mainly subordinated mice.

Elevated plus-maze results (Figure 4) correlated with the data obtained by the previous test. Dominant and subordinate mice showed decreased activity as measured by the number of entries and presence time in the open or closed arms when compared to normal mice (Figure 4A,B). Furthermore, subordinate animals demonstrated a high decreased number of entries into the open arms when compared with the normal and the dominant mice (Figure 4A). Concerning exploratory activity, the time remaining in closed arms was higher for the subordinate mice. These values, in number of visits or time in arms, were similar for subordinate and solitary mice (Figure 4B).

Discussion

Our results, in agreement with data from literature, indicate that groups of male mice in adulthood may present

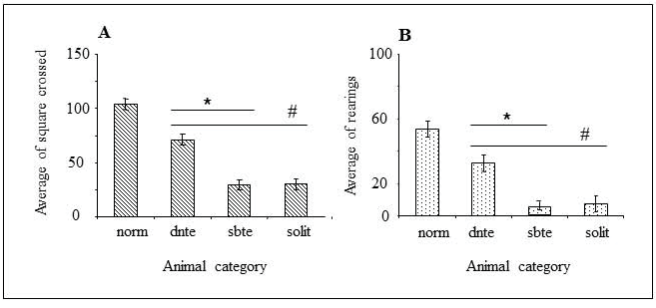

Figure 2: Evaluation of motor and exploratory activity through open field test: We measured the horizontal and vertical displacement in the categories of normal mice (norm), dominant (dnte), subordinates (sbte) and solitary (solit). The horizontal displacement was measured by the average number of quarters crossed by animals in each category (n= 3 or 4/per assay). The vertical displacement was measured by the average number of rearings of animals anywhere in the test arena. (#) indicates a significant difference when dominant, subordinate and solitary mice are compared to normal mice (p ≤ 0,05). (*) indicates a statistical difference when subordinate mice are compared to dominant mice (p≤0,05).

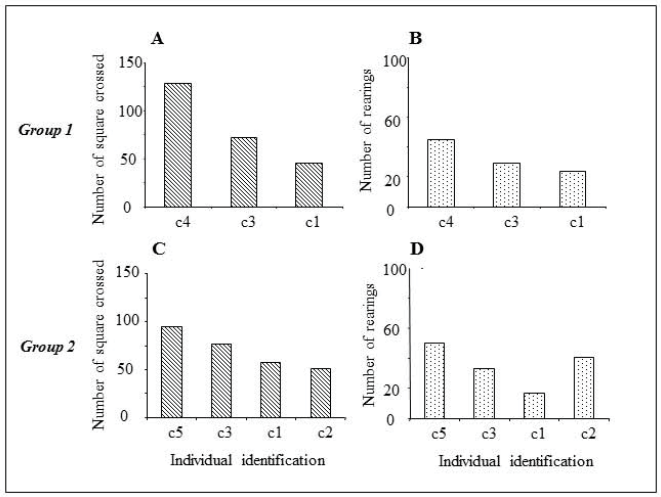

Figure 3: Open field test in dominant mice: We evaluated the motor (A-C) and exploratory (B-D) activity of dominant mice in two groups. In group one, which consisted of four animals, the three dominant animals (c1, c3 and c4 mice) showed different levels of horizontal displacement (A). In the Group 2, composed of four animals, where the various dominant (c5, c3, c2 mice) and subordinated c5 mice showed a decreasing level of activity, suggesting that there is a social hierarchy determined by motor activity. The subordinate mice in each group had lower horizontal and vertical displacement when compared to the dominant mice (data not shown). The number of individual rearings also showed a differentiation, both in the group one (B) and in Group 2 (D), however, the values were not as uniform as those of the motor activity.

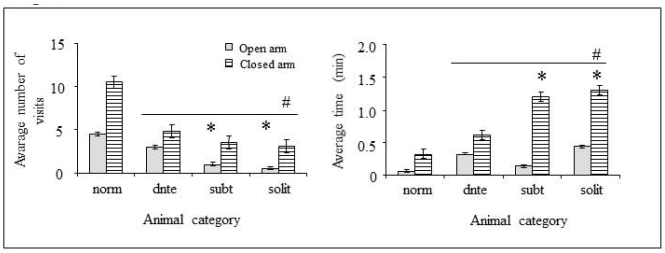

Figure 4: Elevated plus maze test: We evaluated in various categories the average number of entries of mice in the open and closed arms (A). We also measured the average duration of time spent in each area by the animals (B). The subordinate and solitary mice showed a lower number of visits to the open arm and a higher level of time spent inside the closed arm (n= 3 or 4/per assay). (#) indicates statistical difference when dominant, subordinate and solitary mice are compared to normal mice (p≤0,05). (*) indicates a significant difference when the average number of visits in the open arm of dominant and solitary mice are compared to normal and dominant mice (p ≤ 0,05).

PAB [7]. The aggressive behavior can have various causes. The genetic influence may be related to segments of chromosomes with effects on aggressive behavior. This approach is illustrated by the effect of the male-specific part of the mouse Y chromosome on aggressive behavior. It´s proposed that a positional candidate for this effect is Sry [22]. Testosterone also increases adrenal corticoid hormone (ADH) levels in the medial amygdala, lateral hypothalamus, and preoptical medial area, which are involved in aggressive behavior [10]. Neurotransmitter expression, as well as excessive aggressive and impulsive traits of neuronal NO synthase knockout (nNOS-/-) mice were shown to be caused by reductions in serotonin (5-HT) turnover and deficient 5-HT1A and 5-HT1B receptor function in brain regions regulating emotion [23]. We believe that the presence of aggressive behavior and social dominance in adult male mice may be the result of these factors together.

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However, one question still needs to be clarified. Why would the PAB appear in some groups and not others, under the same environmental conditions? Issues such as sexual competition [24] and the inclusion of intruders [25] was described in the literature, but this did not happen in our study. Several studies have described social dominance in male mice and related the description of dominant and submissive categories [26,27].

The motor and exploration activity of the mice is born of the requirement for information related to the new environment. The animal acquires information in two ways, evaluated by testing in the open field and the elevated plus-maze. Horizontal displacement, measured by the number of spaces crossed (motor activity) or displacement in arms (open and closed) in the elevated plus-maze, respectively. Exploratory activity was measured by the number of vertical displacements (rearing) while the animal is anywhere in the arena and time spent in the arms. The results for the two tests were similar. Our results showed that dominant and subordinated mice showed decreased motor and exploratory activity in relation to animals without the PBA, that is, despite presenting different categories, the stress promoted by aggressiveness affects individuals of both categories. So, decrease in activity may be related to the chronic stress caused by the aggressive behavior. Furthermore, subordinate animals showed serious decreased in the activity compared with the dominant mice and similar animals in social isolation, what can we characterize as a state of depression (depression-like) in booth categories [28].

Individual housing has also frequently been reported to be uncomfortable for mice and has even been used as a model for social deprivation in man [29]. It may cause both physiological and behavioral abnormalities, referred to as 'isolation syndrome' [30]. The clinical depression, understanding of the etiology behind these disorders is far from complete, though theories involving various neurotransmitter systems have been advocated [31]. The symptoms - reduced activity of the submissive and isolated animals - suggest a connection to neurotransmitter imbalance similar to the clinical signs of depression in humans [32]. In consequence, these animals show reduced motor and exploratory activity.

Conclusion

We still do not know the cause of the appearance of PAB in some groups of male mice. The high and/or low level of (mainly motor) activity of the animals comes after the process of social dominance or as consequence of this. Are there two types of social dominance that could arise from the activity level of individual mice? Our goal is to propose a model of maintenance of male mice in the laboratory based on their motor and exploratory activity designed to minimize aggressive behavior. We can conclude that animals which have aggressive disorders in their group show alterations in their motor and exploratory activity and similar with isolation social behavior in suggest depression-like condition.

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