Original Article

The development of social dominance is affected by early protein malnutrition in rats.

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ABSTRACT

Early protein malnutrition produces structural and functional alterations in the brain and changes the organism-environment interactions. Rats from 26 to 56 days of age were used to study the effects of early postnatal protein malnutrition on the development of social dominance. During lactation phase the litters were fed diet containing 16% protein (well-nourished) or 6% protein (malnourished). From weaning to the end of behavioral tests well-nourished animals were fed a commercial lab chow diet (well-nourished - W) and the malnourished rats were divided into 2 groups: one was maintained on 6% protein diet (malnourished - M) and the other was fed a commercial lab chow diet (previously malnourished - PM). Pairs of male rats of same diet conditions were tested, at different ages, for three consecutive days. During sessions the pinning behavior (an indicator of social dominance) was recorded. The results showed that both M and PM animals were less efficient to establish social dominance at earlier ages. As M and PM animals turn older they increased social dominance index indicating developmental retards. However both M and PM animals were less able to achieve social dominance index during test sessions (defined as an index of at least 0.75). These results suggest that early protein malnutrition can affect the development of neural mechanisms underlying social dominance in rats. The lower capacity of M and PM animals to establish early social dominance may also suggest possible changes in social interactions at latter ages.

Key Words: Protein malnutrition, social dominance, development, rats.

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INTRODUCTION

The development of social behaviors is a well-organized pattern of vital importance to prepare adequate behaviors to future environmental contingences. It is suggested that the playful behaviors can result in useful strategies later in adult life, preparing the organism to a proficient social behavior on its environment[1,2,3]. Such a suggestion finds support in studies showing that socially isolated animals presented abnormal patterns of agonistic, sexual and social behaviors, stressing the importance of play behavior early in life[3,4,5,6].

Among the various behaviors expressing play the play fighting appears to be the most common in young rats[7]. It has been well established the ontogeny of this behavior with play fighting beginning early in life (around 18 days of age) peaking around 30-40 days of age and decreasing by 50-60 days of age[8,9,10].

Play fighting is believed to have a high incentive value[11,12] and young rats learn instrumental responses to play with another rat[13,14]. Even though an immediate goal of play fighting is difficult to identify, the critical importance of this behavior is evident in the finding that play deprivation leads to significant alterations of behavior later in adult life, such as lower social interaction and aggression[1,15].

Current theories propose that play may be a forerunner of adult aggression[16,17] , and it is through this amicable rough-and-tumble play that young rats learn their place in the hierarchy without sustaining serious injury[18]. Additionally, play fighting may serve to equip the animals with some basic skills and strategies essential for a variety of behaviors that are expressed in adulthood[19,20].

It has been suggested that the analysis of play fighting serves as a useful tool to study the relations of social dominance in rats[21]. The time course of this social dominance shows that it is characterized by a progressive development of asymmetry of play fighting[22]. At the beginning both animals in the pair present the same frequency of initiatives to play. However, an asymmetry rapidly develops and one animal of the pair starts to initiate more of the play fighting. This asymmetry is defined as social dominance[21].

The development of social behaviors can be affected by several environmental conditions. Among these conditions, the nutritional insult is described as one that changes the morphology, neurochemistry and functional aspects of central nervous system[23,24,25], with repercussions on social behavior. It has been shown that both prenatal[26] and postnatal[27] protein malnutrition significantly decrease play behavior in rats. However, increases in play behavior and social responsiveness have also been observed in malnourished rats[28,29,30,31], as well as decrease in social interaction and increase in aggressive behaviors[32]. These divergent results can be due to methodological differences in both the nutritional insult used (malnutrition techniques, time and duration of malnutrition, duration of nutritional recovery) or in the behavioral tests chosen (duration of social contact, age and sex of the animals).

The studies investigating the effects of protein malnutrition upon social behaviors usually measure the play fighting and record only the total number of pins executed by each rat in the pair [26,27]. These data can demonstrate the effects of early malnutrition on the frequency of play fighting but can not show how the social dominance develops across age and across sessions of social contacts. Thus, the main objective of the present work was to investigate the effects of early protein malnutrition on the time course of the development of social dominance in rats aging from 26 to 56 days.

METHOD

Animals

Two hundred forty (120 pairs) of male *Wistar* rats from the animal colony of the Ribeirão Preto Campus, University of São Paulo, were used. During the lactation period (21 days), each litter was culled to 6 males and 2 females on the day of birth. From the same day on, the dams and pups were placed in transparent plastic cages (40 x 30 x 20 cm) and randomly assigned to receive either a 6% or a 16% protein diet *ad libitum*. The two diets were isocaloric and have been described elsewhere[33]. The protein deficient diet contained 6% protein (casein), 5% salt mixture, 1% vitamin mixture, 8% corn oil, 0.2% choline and 77.8% cornstarch. The normal protein diet contained 16% protein (casein), 60.8% cornstarch and the same percentage of the other constituents as the protein-deficient diet. The two diets were supplemented with L-methionine (2.0 g/kg protein) since casein is deficient in this amino acid. After weaning (21 days) the females were discarded and the males were placed in individual plastic cages (30 x 19 x 13cm) and divided in three groups: well-nourished rats (W - 16% protein diet during the lactation phase and commercial lab chow diet from weaning to behavioral tests); previously malnourished rats (PM – 6% protein diet during lactation phase and commercial lab chow diet from weaning to behavioral tests); and malnourished rats (M – 6% protein diet from lactation phase and 6% protein diet from weaning to behavioral tests). During pregnancy the dams were fed a normal protein diet. Independent groups of rats were composed by 7-8 pairs of rats. The rats were maintained on a 12L:12D cycle and room temperature was kept at 23 – 25 °C. Water was available all the time.

Apparatus

The testing arena consisted of an acrylic plastic cage measuring 36 x 36 x 50 cm, with 2 cm of wood shavings covering the floor. A 60 W white light provided the only illumination in the experimental room and was mounted 80 cm above the arena. Experimental sessions were recorded with a vertically mounted video camera linked to a monitor and a video recorder in the adjacent room. The wood shavings covering the floor of the testing arena were changed between tests.

Procedure

To familiarize the animals with the experimental conditions all rats were individually placed in the experimental cage for a 5-min session on each of 5 consecutive days. Test sessions were then held on each of 3 consecutive days and consisted of placing the same pair of rats (non-littermates) of the same age and same nutritional condition in the arena during 10 min. The rats of a pair were selected by weight so as not to differ in body weight by more than 10 g. Independent groups of animals (20 animals per diet condition on each group of age) were tested with 26, 36, 46 and 56 days of age; i.e. rats were not tested at more than one age. For identification purposes the rats were marked with black ink on the top of the head. Videotapes were analyzed by two experimenters blind to the nutritional condition of the rat (i.e., experimenters were not familiar with how group weights are affected by the different diets used) and the pin behavior was recorded. Pin behavior was defined as "one animal lies on its back while the other stands over and in contact with it"[26].

A social dominance index was defined as number of pins of the rat defined as "A" in the pair divided by the number of pins of the rat defined by as "A" plus the number of pins of marked as "B" in the pair as showed bellow.

$$DI = \frac{PA}{PA + PB}$$

Where:
DI = Social dominance index
PA = number of pins of the rat "A" in the pair
PB = number of pins of the rat "B" in the pair

Statistical analysis was conducted with data of the rat of the pair that presented the higher social dominance index (A or B). Social dominance was considered established when one animal in the pair reached the index of 0.75 or more in the third session of play.

Statistical analyses

The body weight of the animals at the test day was analyzed by a one-way ANOVA. Behavioral data were analyzed by three-way ANOVA (diet condition x age x session) with session as a repeated measure. Post hoc analyses were conducted using the Newman-Keuls test. Significance was assumed when $p<0.05$.

RESULTS

Body Weight

ANOVA showed a significant effect of diet on body weight at 26 [F(2,57) = 322.9, p<0.001], 36 [F(2,57) = 688.3p<0.001], 46 [F(2,57) = 1105.9, p<0.001] and 56 [F(2,57) = 527.6, p<0.001] days of age. The animals in the W group weighed more than M and PM animals (p <0.05) in all ages (Table 1).

Table 1

BODY WEIGHT (Mean ± SEM) OF WELL-NOURISHED, PREVIOUSLY MALNOURISHED AND MALNOURISHED RATS DURING DEVELOPMENT

Age (Days)	Experimental Groups		
	Well-Nourished (n=80)	Previously Malnourished (n=80)	Malnourished (n=80)
26	92.00 ± 3.50	37.87 ± 2.73* +	25.85 ± 2.08*
36	148.62 ± 5.01	77.87 ± 3.61* +	32.68 ± 3.01*
46	225.11 ± 4.12	134.12 ± 4.67* +	39.35 ± 3.63*
56	301.87 ± 3.52	204.05 ± 5.12* +	49.47 ± 3.30*

* p<0.05 compared to well-nourished
+ p<0.05 compared to malnourished

Behavioral Measures

Interobserver reliabilities were calculated on 16 random selected pairs of subjects and correlation values (r_s) of $\geq$0.87 were obtained for each of the behaviors recorded.

Data of social dominance index are presented in the Figure 1. Three-way ANOVA indicated a significant effect of diet [F(2,108) = 71.73, p<0.001], with *post-hoc* analysis showing that M animals had lower values of dominance index as compared with C and PM animals (p<0.05). PM animals also had lower values of dominance index as compared with C animals (p<0.05). There was also a significant effect of age [F(3,108) = 121.67, p<0.001], with *post hoc* analysis showing an increase in the social dominance index from 26 to 36 days (p<0.05) and a decrease from 46 to 56 days (p<0.05). ANOVA also indicated a significant effect of session [F(2,216) = 396.55, p<0.001], with post hoc showing increases in the social dominance index across sessions (p<0.05).

Figure 1

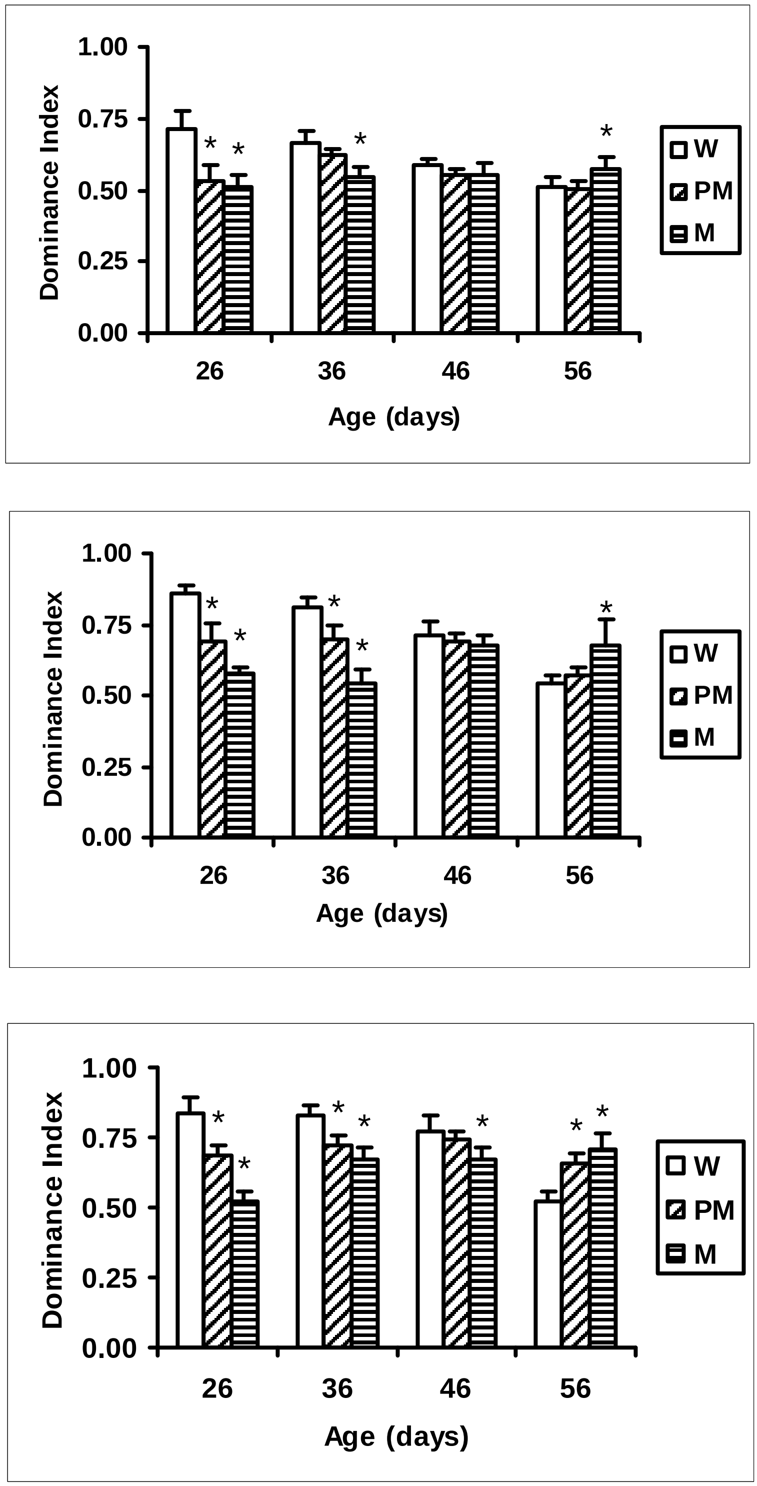

Figure 1. Mean + S.E.M (n = 10 pairs of rats on each nutritional condition and age) of social dominance index on sessions 1, 2 and 3. W = well-nourished rats, PM = previously malnourished rats and M = malnourished rats. * $p<0.05$ compared with W group at the same age.

There was a significant diet x age interaction [F(6,108) = 146.79, $p<0.001$] showing that while W animals decrease social dominance index across age, M animals increase that index and PM animals maintain the index approximating to the W animal values. There was also a significant diet x sessions interaction [F(4,216) = 4.24, $p<0.01$]. Post-hoc analysis showed that animal of all diets conditions increased dominance index across sessions, however the increase was higher for W as compared with PM and M animals ($p<0.05$). ANOVA also showed a significant effect of age x sessions interaction [F(6,216) = 3.67, $p<0.001$] with animals of 26, 36 and 46 days demonstrating higher increases of dominance index across sessions as compared with animals of 56 days ($p<0.05$).

There was also a significant effect of diet x age x session interaction [F(12,216) = 2.97, $p<0.01$]. *Post-hoc* analysis showed that in the first session both M and PM animals had lower dominance index as compared with W animals at the age of 26 days of age ($p<0.05$). On 36 days of age only M animals presented lower dominance index as compared with W animals ($p<0.05$). At the age of 56 days M animals presented higher dominance index as compared with W animals ($p<0.05$). No differences were found ate the age of 46 days. On the second session, the dominance index of M and PM animals are lower as compared with W animals both at 26 and 36 days of age ($p<0.05$). In addition, the dominance index of M animals was higher as compared with W animals at 56 days of age ($p<0.05$). No differences were found in the age of 46 days. Finally, on the third session the dominance index of M animals are lower as compared with W animals on the ages of 26, 36 and 46 days ($p<0.05$), while dominance index of PM animals are lower as compared with W animals only on 26 and 36 days ($p<0.05$). In addition, on the age of 56 days dominance index of both M and PM animals are higher as compared with W animals ($p<0.05$).

DISCUSSION

Early postnatal malnutrition produced a significant reduction in the body weight of animals in the present study. Even the PM animals (undernourished only during the lactation phase) showed significantly lower body weights at 56 days of age, confirming previous reported data in our[33,34,35] and other laboratories[28,36].

Behavioral data indicated that social dominance develops across sessions confirming previous data that play fighting behavior depends of constant social contacts[22,34]. In addition, it was demonstrated in the present study, as well as in previous works (7-10) that the behavior expressing social dominance is more common in young rats (26 and 36 days) as compared with older rats (56 days). However, the contribution of the present study was to demonstrate that is possible to use a social dominance index to show that early protein malnutrition affects the time course of the development of social dominance in rats. It was demonstrated that not only protein malnutrition affects the play fighting but also that it is clearly observed a retard in the development of social dominance in malnourished animals. While W animals present an index of social dominance of 0.75 or more on the second session on ages of 26 and 36 days, as well as on the third session on ages of 26, 36 and 46 days, the M animals did not reach the index of 0.75 established as the criterion to consider that one rat developed social dominance in the test. Although the M animals increased this index across age, while W animals decreased it indicating developmental retard, the M ones did not reach the 0.75 value on none of the ages tested.

It is interesting to observe that PM animals are situated in an intermediary position between W and M animals. These data indicate that nutritional recovery reduced impairment produced by early malnutrition on the development of social dominance; however such a recovery was not sufficient to equalize the index to that demonstrated by W animals. Thus, even after a nutritional recovery period the PM animals demonstrated a long-lasting deleterious effect of early malnutrition on the development of play fighting, an indicator of social dominance.

It has been suggested that play fighting is a forerunner of adult aggression[16,17], and it is through this amicable rough-and-tumble play that young rats learn their place in the hierarchy without sustaining serious injury[18]. Play fighting also serve to equip the animals with some basic skills and strategies essential for a variety of social behaviors that are expressed in adulthood[19,20]. Thus, taken in consideration the role of the play fighting in young rats, then it is possible to suggest that changes observed in social dominance in the present study may disrupt later social behavior in adult malnourished animals.

The data of the present study can also help to understand the earliest studies in the area of malnutrition and social behavior. Those studies already had shown that early malnutrition can change, later in adult life, sexual behavior[37,38], social behavior[29,30], and social dominance[31] of rats, as well as, social behavior[39] and social dominance[40] (40).

The data showing that both malnourished and previously malnourished animals did not developed social dominance across sessions even at 56 days of age can also suggest that those animals may have difficulties to develop necessary skills to maintain social contacts, to mate or even to defend colony territory. This long-lasting impairment of malnourished animals to develop dominance upon another adult male is an interesting data that deserve future investigation to study social behaviors of those animals in procedures of naturalistic environment.

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