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Affiliation in Periadolescent Rats: Behavioral and Corticosterone Response to Social Reunion With Familiar or Unfamiliar Partners

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CIRULLI, F., M. L. TERRANOVA AND G. LAVIOLA. *Affiliation in periadolescent rats: Behavioral and corticosterone response to social reunion with familiar or unfamiliar partners.* PHARMACOL BIOCHEM BEHAV **54**(1) 99-105, 1996. – The aim of this study was to investigate whether the social relationship between periadolescent rats, which is characterized by a high expression of play behavior, resembles attachment or whether it reflects a nonspecific social attraction between peers. Sprague-Dawley rats have been kept in pairs with a conspecific of the same age and sex from the time of weaning (PND 21). On PND 34, the members of each pair were separated for a 24-h period and randomly assigned to one of three groups: a) immediately sacrificed (NT = nontreated); b) reunited for 30 min with their previous cagemate (FAM); or c) with an unfamiliar conspecific of the same age and sex (UNF). During the reunion, the occurrence of both social and nonsocial behaviors was scored. Overall, periadolescent UNF animals spent more time in social investigation than FAM rats, which were conversely more involved in cage-oriented activities. In addition, higher levels of rough-and-tumble play were expressed in encounters between UNF subjects than between FAM rats. Finally, within the female group, UNF rats were more involved in play soliciting and less in digging activity than FAM animals. Blood corticosterone levels, which were measured at the end of the separation period, were higher in females than in males and were significantly lower than following reunion in a novel environment. The degree of familiarity did not affect hormonal changes. Results suggest that periadolescent rats do not form attachment relationships with each other. However, partner familiarity appears to be an important variable affecting social relationships.

Periadolescence Social interactions Play Familiarity Corticosterone Novelty

IN the rat, periadolescence encompasses the 7-10-day period prior to the onset of puberty at about 40 days and the first few days thereafter (34). Periadolescent rats differ markedly from slightly younger and older animals in several aspects of behavior and physiology, as well as for the behavioral response to psychoactive drugs (11,22,34,44). They also exhibit elevated levels of affiliative and playful social interactions among peers, mainly consisting of rough-and-tumble play [(24,31,40,42); see also (38) for the mouse]. It has been hypothesized by several authors that such a particular behavioral profile may be adaptive for the specific ecological niche of periadolescent animals, facilitating the expression of exploration and sociality (6,15,16,23,25).

The processes that underlie the high propensity of periadolescent rats to show social interactions with conspecifics have not been clarified yet. In this respect, two working hypotheses can be proposed. The first one is that social interactions typical of this developmental stage reflect the existence of specific social bonds between familiar partners, similar to those that characterize mother-infant relationships in primates or mate relationships in monogamous species (8,17). A second possibility is that affiliative and play behaviors shown by periadolescent rats rely upon a nonspecific social attraction between same-age conspecifics.

Attachment behavior has been described as any form of behavior that results in a subject attaining or retaining proximity to some other differentiated and preferred individual (7). Studies conducted in nonhuman primates have shown that separation from an attachment figure, such as the mother for an infant monkey, results in intense agitation, with rapid

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movements and frequent isolation calls. In both members of the dyad, this behavioral response to separation is paralleled by a marked increase in plasma concentrations of cortisol. However, upon reunion, cortisol levels decrease, while there is a temporary increase in attachment behavior, measured as the amount of time animals spend in contact (9,35).

In rodents characterized by a monogamous social system, such as praire voles, separation of long-term heterosexual pairs for 24 h increases the activity of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in higher corticosterone (CORT) levels (8,43). However, reunion with a familiar partner at the end of the separation period results in a significant decrease in CORT levels, whereas the levels of this hormone are still high following reunion with an unfamiliar conspecific. Even if no behavioral data are available for these animals, the above results support the hypothesis that at least in this rodent species attachment underlies their social relationships.

With respect to our second hypothesis, other than attachment, the relationship between periadolescent rats could reflect a general social attraction between same-age conspecifics, which does not require previous familiarity. However, in the literature, there are surprisingly few and contrasting results regarding the effects of partner familiarity on rat affiliative behavior during this ontogenetic stage. A first study, carried on in periadolescent rats by Latané and colleagues (21), showed no differences in social proximity, a measure of gregariousness, in male subjects tested for 4 consecutive days with either the same partner or a different one each day. On the contrary, Barefoot and co-workers (4), as well as Monroe and Milner (27), using similar methodologies, found that affiliation was increased in those rats paired with a different conspecific on each test day, concluding for an important motivating role of partner novelty [see also (39)]. However, no data are available on the adrenocortical activity of periadolescent rats following separation and reunion with conspecifics.

In adult rats, a few studies have assessed both behavioral and adrenocortical responses to social reunion with familiar or unfamiliar partners. These reports show that separating the members of a group does not affect their HPA axis activity, suggesting that adult rats do not show attachment to each other (2,3,12,14). Group-housed rats, however, do show some kind of recognition of their cagemates because familiarity affects both their adrenocortical responses and social behavior when they are placed together in pairs. In fact, time spent in social interaction is higher, while adrenocortical activation is lower, in unfamiliar compared to familiar rats (2,3). Thus, differently from the praire voles, another rodent species showing attachment relationships, adult rats seem to interact preferentially with an unfamiliar conspecific, rather than a familiar one.

However, the above results were not obtained by testing established rat pairs, but in adult animals kept in groups, and one could expect that, compared to grouped animals, the two members of a pair might develop a greater cohesion. Furthermore, because of what already said about affiliative social interactions during periadolescence, the social behavioral repertoire exhibited during this developmental period differs qualitatively and quantitatively from that of adult animals.

The aim of the study was to investigate whether the kind of social interaction between pairs of periadolescent rats resembles a nonspecific social attraction or whether it reflects the presence of a specific bond. To address this issue we analyzed both hormonal and behavioral responses to reunion between same-sex pairs of periadolescent rats following 24 h of social isolation.

If the relationship between the members of a periadolescent rat pair was similar to attachment, we might expect CORT levels to be significantly decreased only upon reunion with a familiar partner. In addition, a higher level of social interaction would be expected when reuniting familiar as compared to unfamiliar rats. If, on the other hand, periadolescent rats were attracted to each other only by an age-related nonspecific need for social interaction, familiar and unfamiliar partners should have similar behavioral and hormonal responses following reunion. Alternatively, the novelty of the partner might have greater attraction properties than familiarity (4,27), so that one might even expect unfamiliar rats to show higher levels of social interactions, and lower CORT levels, than familiar pairs.

METHOD

Animals, Breeding, and Rearing Conditions

Sprague–Dawley pregnant rats were obtained from Charles River Italia (Calco, Italy). Upon arrival, animals were maintained in an air-conditioned room at 21 ± 1°C and 50 ± 10% relative humidity and housed separately in 40 × 25 × 20 cm Plexiglas boxes, with sawdust as bedding and a metal top. The animals were housed in a 12 L : 12 D cycle with lights on at 0930 h. Food (enriched standard diet purchased from Piccioni, I-25100, Brescia, Italy) and water were freely available.

From weaning (PND 21) until the time of testing, each pup was kept together with a same-sex conspecific in a 33 × 13 × 14 cm Plexiglas box. On PND 34, members of all pairs were weighed and housed individually in a clean cage of the same size as the home cage for a 24-h period. At the end of the 24 h isolation period: a) subjects in the NT group were sacrificed and their trunk blood collected; b) FAM subjects were reunited with their previous same-sex cagemate, i.e., with a familiar partner for 30 min; c) UNF subjects were reunited for 30 min with an unfamiliar same-sex conspecific. Immediately after the social encounter, both FAM and UNF rats were sacrificed and blood samples collected.

Age of testing was chosen taking into account literature data on periadolescent rats showing that rough-and-tumble play, which represents the most important and pervasive kind of social interaction in developing rats, peaks around PND 35 (see the introductory paragraph).

Blood Samples

Animals were killed by decapitation and trunk blood collected in heparinized tubes. Blood samples were centrifuged at 2000 rpm for 20 min to obtain cell-free plasma, and then frozen at −80°C until they were assayed for CORT with a radioimmunoassay kit (ICN Biochemicals, Costa Mesa, CA; sensitivity = 0.125 mg/dl).

Behavioral Observations

FAM and UNF social encounters lasted 30 min, and took place under white-light in (21 × 27 × 14 cm) Plexiglas boxes placed in an experimental room that was maintained at the same conditions as the housing room [for the use of the social interaction test, see (13,28)]. The box was novel to both individuals, of a different size than the home cage and, in addition, it was bedded with clean sawdust. Behavioral testing took place from 1000 to 1400 h, and the time of testing counterbalanced between experimental groups.

The behavior of each pair was videorecorded by means of two videocameras connected to a professional Sony videocassette recorders V0-5800PS. We scored separately the first (min 1-10) and the last 10 (min 10-20) min of the encounters to compare behavioral differences as a function of time and to measure the development of a habituation profile.

Behavior was scored by means of a keyboard event recorder system feeding to a computer for analysis. The Observer, a software system for collection and analysis of observational data, was used for scoring duration and frequency of each response (29). A focal animal—all occurrences sampling method (1) was used, with each animal being continuously observed for randomly chosen 5 min in each of the two 10-min periods. Because the behavior of one of the two subjects in a pair cannot be considered independent from that of the other, pair means were used for further analysis.

The social and nonsocial behavioral elements listed below, and their classification, are mainly based upon the ethological profiles of rat behavior described by Poole and Fish (34), Meaney and Stewart (24), Panksepp and co-workers (31), and Thor and Holloway (41) [see also (36,38)].

Unless otherwise specified, both frequency (f) and duration (d) were measured for each behavioral item.

Behavioral Categories

Social activities. A—Investigative elements: 1) Social Sniff: self-explanatory (s.e.); 2) Follow: (s.e.); 3) Mutual Circle: partners are mutually sniffing each other's anogenital region, while describing tight circles with their reciprocal following movements.

B—Affiliative elements: 1) Social Inactive: a rat is lying flat or standing still (eyes closed or open) while maintaining close physical contact with the partner; 2) Social Rest: is being groomed by the partner; 3) Allogroom: s.e.

C—Play soliciting: 1) Push under (f): pushes its own snout or the whole anterior part of its body under the partner's body, and then rests; 2) Push past (f): passes between the wall of the cage and the body of the partner by pushing its own body through the narrow space available; 3-4) Crawl under/over (f): crawls underneath/over the partner's body, crossing it transversely from one side to the other.

D—Rough-and-tumble play: the two rats exhibit sudden darting movements, which are associated with pouncing on the partner back, chasing, wrestling and pinning (the so-called on-top and on-back postures). The play bout is often anticipated by a prolonged and intense bout of allogrooming, often accompanied by gross movements of the whole body of the performer and by vigorous pulling of the fur of the partner. The bout is considered concluded when both participants either turn their attention away from each other, or shift to amicable nonplayful interactions (e.g., sniffing).

This measure was preferred to the scoring of the sole pin behavior, which is frequently used as a reliable and time-saving method to quantify play behavior [e.g., (31)]. In fact, our behavioral scoring was aimed at a global evaluation of the social repertoire that characterises periadolescent rats. Therefore, we had to consider all its different components, which can or can not be related to play, namely rough-and-tumble play, play-soliciting, affiliative, and investigative social behaviors [see also (42)].

Nonsocial activities. A—Activity and Exploration/Escape behaviors: 1) Inactive: (s.e.); 2) Explore: (s.e.); 3) Jump (f): jumps up vertically to a wall, often repeatedly, with the snout directed upward; 4) Dig: is digging in the sawdust, pushing and kicking it around using the snout and/or both the forepaws and hindpaws, mostly moving around the cage and sometimes changing the whole arrangement of the substrate material.

The functional significance of elements 3 and 4 probably includes both exploration and escape components.

B—Maintenance activities: 1) Selfgroom: (s.e.).

Design and statistical analysis Behavioral data were analyzed by means of a 2 × 2 × 2 mixed-model ANOVA, with familiarity (FAM vs. UNF) and sex as between-subject factors, and time (min 1-10 vs. 20-30) as within-subject factor (repeated measures). Corticosterone data were analyzed by means of a 2 sex (male vs. female) × 3 treatment (NT vs. UNF vs. FAM) ANOVA. Mean comparisons were carried out wherever significant interaction effects were detected by the ANOVA.

RESULTS

Social and Nonsocial Behaviors

The elements mutual circle, crawl under, inactive, and jump were expressed too rarely and were, therefore, discarded from the ANOVA. When both frequency and duration were scored, the results for the two measures were consistent with each other and, therefore, for the sake of space, only the results regarding the latter are reported. Because the analysis of Dig duration did not yield significant results, only frequency data are reported for this behavior.

Time effects. The comparison between the first and the last 10 min of the encounter showed a marked habituation of both nonsocial and social investigation, whereas the expression of affiliative and playful behaviors increased with time.

In particular, as shown in Figs. 1 and 2, a marked habituation occurred for the duration of cage exploration [time: $F(1, 56) = 45.03, p < 0.001$], and for items of social investigation such as sniff and follow [time: $F(1, 56) = 25.69, p < 0.001$, and $F(1, 56) = 5.427, p < 0.05$, respectively].

Conversely, periadolescent rats appeared to spend more time in Social Inactive, Social Rest, and Allogroom in the last 10 min compared to the first time interval [time: $F(1, 56) = 6.111, p < 0.01$; $F(1, 56) = 11.57, p < 0.001$; and $F(1, 56) = 14.42, p < 0.001$, respectively] (see Fig. 2). As shown in Fig. 3, a similar tendency was also evident for rough-and-tumble play [time: $F(1, 56) = 3.254, p < 0.07$].

The frequency of push under, a soliciting item, showed a marked habituation during the session [time: $F(1, 56) = 4.256, p < 0.05$; data not shown], whereas rats tended to devote more time to maintenance activities as the session progressed [self-groom, time: $F(1, 56) = 3.617, p < 0.06$; data not shown].

Familiarity effects. A clear-cut difference emerged between the behavioral repertoire expressed by FAM and UNF pairs. UNF rats were found to be more involved in investigative and playful social interactions than FAM animals. In particular, they showed an enhanced expression of investigative behaviors such as sniff [familiarity: $F(1, 56) = 6.332, p < 0.01$], whereas only a trend was found for follow [familiarity: $F(1, 56) = 2.816, p < 0.09$] (see Fig. 2). UNF rats proved to engage in rough-and-tumble play for longer periods of time compared to FAM animals [familiarity: $F(1, 56) = 3.782, p < 0.05$] (see Fig. 3), also showing an increased frequency of a soliciting item such as push under [familiarity: $F(1, 56) = 4.256, p < 0.05$] (see Table 1).

As for explore, FAM rats showed a greater intrasession habituation, compared to UNF subjects [familiarity × time: $F(1, 56) = 5.743, p < 0.01$] (see Fig. 1). In particular, during

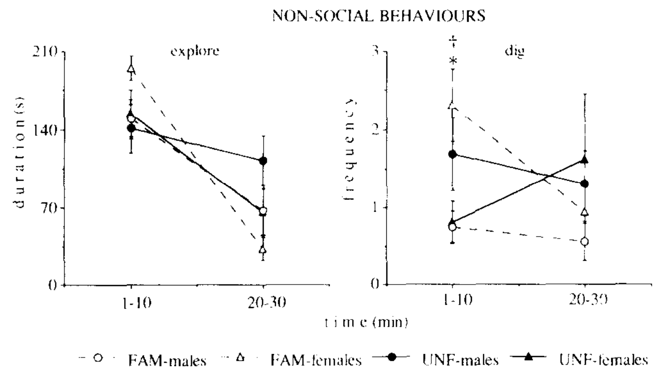

FIG. 1. Mean values (±SEM) of nonsocial behaviours recorded during social encounters between same-sex pairs of males and females periadolescent rats of the FAM and the UNF group ($n = 8$ pairs). Both behavioural items were scored over the first and the last 10-min period (total duration of the session: 30 min). *$p < 0.01$, males vs. females within the FAM group; †$p < 0.05$, FAM vs. UNF within the females group.

the last time interval FAM animals spent a significantly lower amount of time exploring the cage than UNF rats ($p < 0.05$).

Interestingly, at the end of the session FAM pairs showed a tendency to be more involved in affiliative behaviors such as social inactive, social rest, and allogroom than UNF animals (familiarity × time: ps = NS) (see Fig. 2).

Sex effects. In the absence of any significant main effect of the sex variable, it was found that, as a whole, males spent more time than females in cage exploration (explore) as the session progressed [sex × time: $F(1, 56) = 6.681, p < 0.01$], also showing a less marked intrasession habituation (see Fig. 1).

For some behavioral elements, the sex of the subjects interacted with the effects of familiarity. In fact, a significant interaction of familiarity and sex, $F(1, 56) = 3.859, p < 0.05$, as well as an almost significant higher interaction involving time [familiarity × sex × time: $F(1, 56) = 3.483, p < 0.06$] (see Fig. 1) indicated a difference in the frequency of dig between sexes within the FAM group ($p < 0.05$), which was particularly evident at the beginning of the session ($p < 0.01$). In addition, within the female group, and again at the beginning of the session, a significant difference appeared, in that FAM subjects exhibited an increased expression of dig compared to the UNF ones ($p < 0.05$). This difference was no longer there

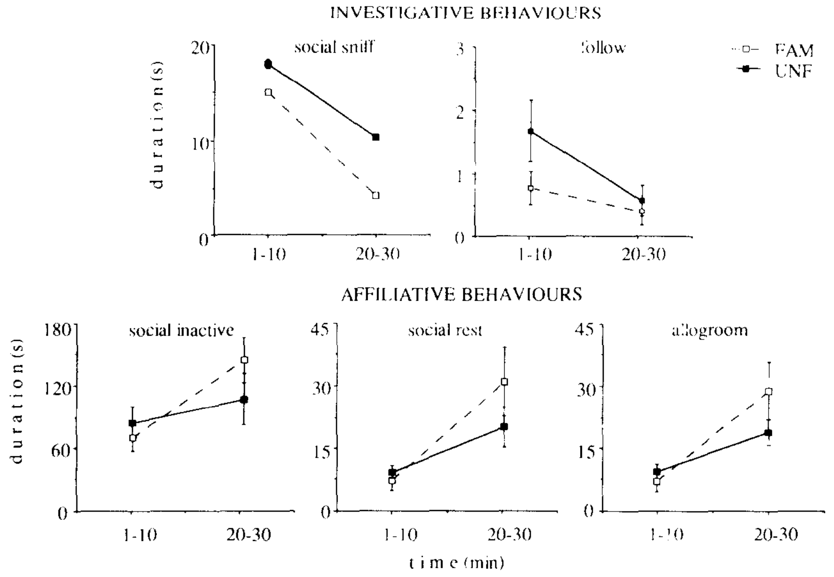

FIG. 2. Mean duration (±SEM) of social behaviours (investigative and affiliative items) recorded during the first and the last 10-min period (total duration of the session: 30 min) in social encounters between same-sex pairs of periadolescent FAM and UNF rats ($n = 16$ pairs). Data refer to the same animals of Fig. 1.

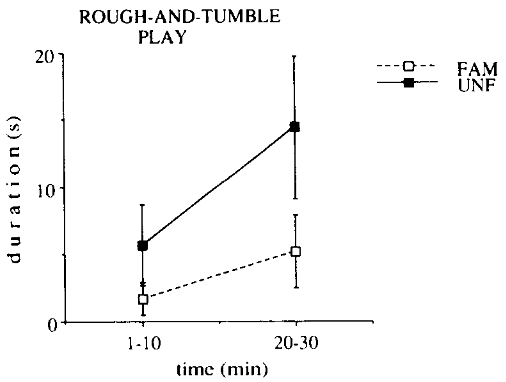

FIG. 3. Mean duration (±SEM) of rough-and-tumble play recorded during the first and the last 10-min period (total duration of the session: 30 min) in social encounters between same-sex pairs of periadolescent FAM and UNF rats ($n = 16$ pairs). Data refer to the same animals of previous table and figures.

at the end of the session, since FAM females showed a marked intrasession habituation ($p < 0.05$). Within the male group, the overall frequency of dig observed in FAM rats tended to be lower than the one shown by UNF animals ($p < 0.06$).

Finally, while within the male group FAM rats showed a higher frequency of crawl over compared to UNF animals (see Table 1), the opposite was true for females [familiarity × sex: $F(1, 56) = 8.881, p < 0.01; p < 0.05$ for both comparisons]. UNF females also expressed a greater amount of crawl over than UNF males ($p < 0.01$).

Corticosterone

Data on corticosterone secretion are shown in Fig. 4. The ANOVA revealed a main effect of sex, $F(1, 69) = 6.5, p < 0.01$, with females showing overall higher corticosterone levels than males. Corticosterone levels also differed significantly among the different treatment groups, $F(2, 69) = 30.12, p < 0.001$. In particular, hormonal levels at the end of the 24 h of social isolation were significantly lower than those found at the end of the 30 min reunion with both FAM and UNF subjects ($p < 0.01$) and did not differ between sexes. Interestingly, although the sex by treatment interaction failed to reach statistical significance, CORT levels of FAM females tended to be higher compared to those of UNF females.

TABLE 1

MEAN FREQUENCY (±SEM) OF PLAY-SOLICITING BEHAVIORS RECORDED IN SOCIAL ENCOUNTERS BETWEEN SAME-SEX PAIRS OF PERIADOLESCENT FAM AND UNF RATS

	FAM	UNF
Crawl over		
Males	0.78 (±0.19)	0.25 (±0.31)*†
Females	0.31 (±0.12)	0.93 (±1.19)*
Push under	0.09 (±0.04)	0.26 (±0.07)‡

($n = 8$ pairs for crawl over, 16 pairs for push under).

In the absence of Sex effects, data for Push under are pooled across sexes.

*$p < 0.05$, FAM vs. UNF within the male group.

*$p < 0.05$, FAM vs. UNF within the female group.

†$p < 0.01$, females vs. males within the UNF group.

‡$p < 0.05$, FAM vs. UNF.

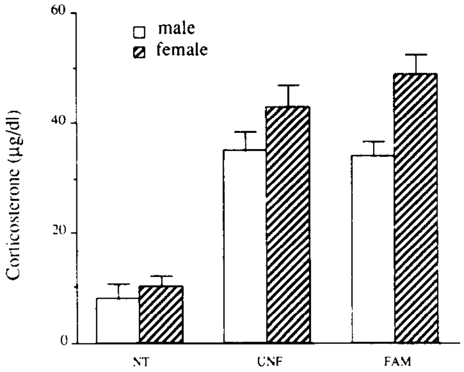

FIG. 4. Mean corticosterone levels (±SEM) in male and female periadolescent rats kept in pairs with a same-sex conspecific from weaning. NT = subjects were isolated for 24 h and blood sampled at the end of the isolation period; UNF = subjects were reunited for 30 min with an unfamiliar conspecific after 24 h of isolation and sampled at the end of the reunion; FAM = subjects were reunited for 30 min with their previous cagemate after 24 h of isolation and sampled at the end of the reunion ($n = 8$ subjects for NT, 16 subjects for FAM and UNF).

DISCUSSION

Overall, data from the present experiment indicate that the behavioral repertoire of periadolescent rats is markedly affected by familiarity. In fact: i) UNF pairs showed higher levels of social investigation and play behavior, whereas they tended to spend less time in passive contact and allogrooming, compared to FAM rats; ii) the sex of the subjects interacted with familiarity, with FAM females showing higher levels of dig, a behavior reflecting emotional arousal (see below), while exhibiting a lower frequency of play soliciting, compared to the corresponding UNF animals; iii) with respect to neuroendocrine measures: a) as expected, female rats showed higher CORT levels than males; b) following a 30-min reunion in a novel environment, CORT levels were increased in both the FAM and the UNF group; c) familiarity did not affect significantly CORT levels.

As far as our initial hypothesis is concerned, periadolescent rats do not show attachment relationships with each other. In fact, when the members of a pair were reunited following a 24-h separation period, corticosterone levels did not differ as a function of partner familiarity. These data are in contrast with reports indicating that in monogamous species of rodents the members of a familiar pair show lower CORT levels, upon reunion, compared to unfamiliar subjects (43).

In contrast to the hormonal parameters, familiarity exerts a strong effect on the behavior shown by periadolescent rats placed in a novel environment. In fact, the members of FAM and UNF pairs acted differently towards each other upon reunion. On the one hand, FAM rats showed a tendency to spend more time in passive physical contact (social inactive and social rest) and allogrooming than UNF subjects. These two last behavioral elements are usually expressed in the context of an established relationship, and are increased in attachment relationships following separation and reunion (30,35). On the other hand, UNF partners engaged more in active social interactions than FAM rats. Thus, our data suggest that during the 14 days of pair housing the two cagemates had developed some kind of a relationship. However, they also indicate that the novelty of the stimulus partner may possess greater attraction properties than familiarity.

In fact, UNF subjects of both sexes were significantly more involved in investigative behaviors, such as sniff and follow, and in soliciting behaviors, such as push under, as well as in more rough-and-tumble play than the FAM group. According to previous findings suggesting that partner novelty has an important motivating role in social behavior, a novelty or curiosity factor may be involved in such an increased gregariousness [(4,27); see also (39)]. Moreover, the presence of a conspecific, and particularly of a novel and, therefore, more interesting one, may be hypothesized to act as a distracting stimulus, reducing the stress associated with the exposure to a novel environment.

Previous studies have examined the effects of social variables on the pituitary-adrenal activity in both nonhuman primates and rodents. For example, it has been shown that the presence of a social group can ameliorate the neuroendocrine response to aversive stimuli that normally evoke an increase in adrenocortical activity in individually housed subjects (26). However, it has also been shown that presence of an unfamiliar conspecific can be more effective than that of a familiar one in reducing the HPA axis activity during novelty exposure (18). In fact, plasma cortisol levels of female monkeys exposed to a novel environment with a familiar partner were found to be similar to those of females exposed to that environment individually. By contrast, the presence of an unfamiliar conspecific attenuated the increase in cortisol levels resulting from the novelty (18). A number of studies conducted in rodents have also demonstrated the ability of social variables in modulating the adrenocortical response to a novel situation. Armario and colleagues [(2,3); see also (12) and (14)] used various behavioral and hormonal parameters to analyze the effects of partner novelty on fear experienced by rats placed in stressful situations in pairs. They found that the corticoadrenal response to a novel environment was higher in pairs of familiar rats than in pairs of unfamiliar ones or in animals tested alone. As for behavioral measures, unfamiliar animals were found to spend more time than familiar ones in active social interactions, while the opposite was true for defecation, ambulation, and rearing (3). While the present behavioral data confirm the above results obtained with adult rats, familiarity with the partner did not affect CORT secretion in response to a novel environment. These differences might perhaps be accounted for by age-related changes in adrenocortical secretion because periadolescent rats, instead of adult subjects, were used in this study. Furthermore, in this experiment the effects of novelty cannot be separated from the effects of exposure to the partner. We, thus, cannot exclude that the stress represented by the novel environment produced a maximal adrenocortical response and that this masked possible differences between the FAM and the UNF groups.

It seems, however, interesting to compare the hormonal data with the behavior recorded during social reunion, particularly with respect to gender differences. In fact, higher frequencies of dig, a response that has been associated with emotional arousal [e.g., (37,38)], were expressed by FAM females compared to UNF females, while no corresponding difference was found in the male group. In addition, while crawl over, a play soliciting item, was expressed at a higher level by UNF females compared to FAM females, FAM and UNF males did not differ in this measure. Indeed, such a lower emotional response to the novel environment and the greater tendency to interact with the partner could perhaps be associated with a somewhat lower adrenocortical output found in UNF females compared to FAM females. Both these responses were absent in the male group.

Clear sex differences have been reported in the baseline of anxiety-related behaviors (19), and female rats appear to be less emotional than male rats in response to novelty (5,10). Furthermore, male and female rats show characteristic differences in corticosterone secretion, with females secreting more CORT than males (20). While in the present experiment females showed significantly higher CORT levels than males, clear-cut sex differences in social and playful behavior were not detected, apart from a female primacy in a soliciting item (crawl over), which only appeared within the UNF group. These findings need to be viewed in the light of the controversial issue of rat sex differences in play behavior [e.g., (24,31, 34,41)]. In this respect, methodological differences should be taken into account, including rearing conditions and observational procedures [see Discussion in (41) and in (31)]. However, some sex differences emerged when comparing the behavior of male and female rats at the beginning and the end of the social encounter. Males spent more time in cage exploration than females during the last 10 min of observation, thus showing a less marked intrasession habituation than females for this behavioral item. Female rats are reported to explore more than males if exposure to novelty is short, but with longer exposure times males gradually increase their exploration level and surpass the females (33). In agreement with the literature, both nonsocial and social investigation decreased over the session [e.g., (39)], while affiliative and playful interactions increased. We cannot exclude the possibility that, given enough time, unfamiliar males might have shown more social interactions.

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