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Long-term social recognition memory in adult male rats: factor analysis of the social and non-social behaviors

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Abstract

A modified version of the intruder-resident paradigm was used to investigate if social recognition memory lasts at least 24 h. One hundred and forty-six adult male Wistar rats were used. Independent groups of rats were exposed to an intruder for 0.083, 0.5, 2, 24, or 168 h and tested 24 h after the first encounter with the familiar or a different conspecific. Factor analysis was employed to identify associations between behaviors and treatments. Resident rats exhibited a 24-h social recognition memory, as indicated by a 3- to 5-fold decrease in social behaviors in the second encounter with the same conspecific compared to those observed for a different conspecific, when the duration of the first encounter was 2 h or longer. It was possible to distinguish between two different categories of social behaviors and their expression depended on the duration of the first encounter. Sniffing the anogenital area (49.9% of the social behaviors), sniffing the body (17.9%), sniffing the head (3%), and following the conspecific (3.1%), exhibited mostly by resident rats, characterized social investigation and revealed long-term social recognition memory. However, dominance (23.8%) and mild aggression (2.3%), exhibited by both resident and intruders, characterized social agonistic behaviors and were not affected by memory. Differently, sniffing the environment (76.8% of the non-social behaviors) and rearing (14.3%), both exhibited mostly by adult intruder rats, characterized non-social behaviors. Together, these results show that social recognition memory in rats may last at least 24 h after a 2-h or longer exposure to the conspecific.

Key words: Agonistic behavior; Long-term social recognition memory; Non-social behavior; Principal component analysis; Social behavior; Social investigation

Introduction

Recognition of a conspecific is advantageous because members of the group can spend time on activities related to group management and protection rather than on vigorous investigation of a previously met non-hazardous individual. Social memory refers to the ability of animals to change their social behaviors towards a conspecific as a consequence of a previous social encounter with it. Social recognition memory in rodents seems to be based mainly on olfactory cues (1-5).

A popular behavioral model used to assess rodents' social recognition memory in the laboratory is the intruder-resident paradigm (6-11). Briefly, when an intruder conspecific is introduced within a resident male rat's home cage for a 5-min encounter, the resident rat exhibits intense social investigation activity towards the intruder. The introduction of the familiar intruder within the resident's home cage for another 5-min encounter elicits far less social investigation compared to that which occurred during the prior encounter and that seen towards a novel intruder. However, this effect is only seen when the intertrial interval (ITI) is about 30-60 min but not when it is about 2 h (1,5,7,10); that is, at longer ITIs, the effect vanishes. This decreased social investigation during the second encounter with a familiar intruder, associated with a lack of decrease in social investigation towards a different intruder, is taken as evidence of social recognition memory. The intruder-resident paradigm has been used as a research tool to investigate short-term memory in rats (5,7,10-12), and may potentially provide an animal model for investigating social interaction dysfunctions such as autism (13).

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There have been attempts to increase the duration of social recognition memory in this behavioral task (6,7). For instance, by using two successive 5-min exposures to the intruder, Dantzer et al. (7) demonstrated consistent social recognition memory when testing was performed 2 h after the last exposure. Similarly, when Sekiguchi et al. (6) tested the effects of either one 30-min exposure or six 5-min exposures to the familiar intruder with ITIs of 10 min and tested the animals 24 h later, their results showed that social recognition memory had vanished for both groups.

Burman and Mendl (14) group-housed four juvenile female rats for 18 days. These subjects were then isolated for 1, 48, or 96 h and then simultaneously exposed to odor cues originating from unfamiliar rats and from former cage-mates. The juvenile female rats spent significantly more time investigating the unfamiliar odor when their isolation period was 1 and 48 h, but not 96 h, indicating that they remembered former cage-mates for at least 48 h.

The present study employed a modified version of the intruder-resident paradigm to investigate the effects of increasing time exposure to an adult intruder conspecific on the social recognition memory evaluated 24 h after the end of the first encounter. Independent groups of rats were exposed to an intruder conspecific for 0.083 (5 min), 0.5 (30 min), 2, 24, or 168 h and tested 24 h after the end of the first encounter with either the familiar or a different adult intruder conspecific. Social and non-social behaviors of both resident and intruder rats were separately scored. Furthermore, a factor analysis was employed to identify possible associations between scored behaviors and their relationships with the treatments. The results clearly show that social recognition memory in rats may last at least 24 h. It was also shown that detailed analysis of the behavioral scores may contribute to a better understanding of the social relationships established during performance of the intruder-resident paradigm.

Subjects and Methods

Subjects

A total of 146 naive male Wistar rats (*Rattus norvegicus*) from the Biosciences Institute colony, about 12 weeks old at the beginning of the experiments, were used. Groups of 4-5 rats were maintained in the same cage (40 x 32 x 16.5 cm) with fresh bedding. Light was provided from 6-18 h, and room temperature was maintained at 21 ± 3°C. Food and water were available *ad libitum*. Animals were handled individually 5 min per day for 2 days before the beginning of the experiments in order to reduce manipulation-induced stress. Since rats exhibit more social behaviors during their inactive phase (15), all experiments were run from 8:00 to 11:00 h. Half the rats were used as intruders and the other half as residents (see below); these social roles were assigned at random. During the experiments, it was ensured that residents and intruders had never shared a cage before. All procedures described respected the Institute guidelines about animal experimentation, which comply with national and international rules and policies (see www.mct.gov.br and www.oacu.od.nih.gov).

Behavioral procedure

Behavioral tests were run in a 39 x 32 x 18-cm Plexiglas cage with fresh bedding. A video camera (Sony CCD, China) positioned 30 cm behind a transparent cage wall allowed tape recording of the social interaction.

A modified version of the intruder-resident paradigm (for review, see Ref. 16) was used. One hour before testing started, the animals were transported from the animal facility to the testing room. The so-called residents were then placed individually inside the testing chamber 20 min before the introduction of an adult intruder conspecific within the same chamber. Adult conspecifics were used as intruders in order to provide the resident rats with a more significant social stimulus (see Ref. 16, for studies involving mice). Because an adult intruder represents a potential territorial competitor for the resident rat, this should increase social recognition memory of the latter rat. This first encounter lasted 0.083 h (5 min; N = 14), 0.5 h (30 min; N = 14), 2 (N = 16), 24 (N = 15), or 168 h (N = 14), depending on the group. At the end of this encounter, the intruder rat was removed and placed in another cage and residents remained within the same testing chamber for 24 h. During this time, residents and intruders were maintained on opposite sides of the animal facility. One hour before the second encounter, rats were transported to the experimental room. During the second social encounter, the residents were exposed to either the familiar (same) or a different (unfamiliar) intruder for 10 min. Residents and intruders were exposed either to the familiar or to a different resident during the second encounter, and rats exposed to different conspecifics in the second encounter had similar prior experiences with a conspecific in terms of duration of the first encounter (i.e., intruders whose first encounter lasted, for instance, 2 h were exposed to unfamiliar residents exposed to an intruder for 2 h).

The second encounter was video recorded, allowing scoring of the time spent by both the resident and the intruder rats performing social or non-social behaviors, defined according to previous studies (see Refs. 4,7 9) and according to the most frequent behaviors observed during social encounters (Moura PJ, unpublished data). Social behaviors included 1) sniffing the conspecific's anogenital region (ANO), 2) sniffing the conspecific's head (HEAD), 3) sniffing the conspecific's body (BODY), 4) following the conspecific (FOLL), 5) dominance behavior (DOM; corresponding to handling the conspecific whose back is either on the floor or against the wall), and 6) mild aggression (AGGR; to beat using the legs); non-social behaviors included 7) sniffing the environment (ENV), 8) self-grooming (GROOM), and 9) rearing (REAR; see Table 1) (3,6,7,11,17,18). No biting attacks or any other aggressive behavior that could injure

the conspecific were observed. A home-made computer-assisted data acquisition system allowed an experimenter blind to the treatments to score each individual behavior during four time bins of 150 s each; the successive time bins corresponded to the time intervals from 0 to 150, 150 to 300, 300 to 450, and 450 to 600 s.

Data analysis

The total time spent by each rat exhibiting social and non-social behaviors was calculated for each time bin and also for the complete second encounter. Furthermore, the relative percentage of time spent performing each scored behavior relative to the total time of its corresponding category (social or non-social) was also calculated for each rat. For representational purposes, a general mean, including both resident and intruder rats exposed to all experimental conditions, was calculated. Finally, taking into account the results of the principal component analysis (PCA; see below), it seemed relevant to subdivide social behaviors into two categories. Thus, we calculated the total time each rat spent performing either 1) social investigatory behaviors, which corresponded to the sum of time spent performing ANO, HEAD, BODY, and FOLL, or 2) social agonistic behaviors, which corresponded to the sum of times spent performing DOM and AGGR. Even though the social behaviors and their subcategories, social investigatory and social agonistic behaviors partially overlap, it seemed relevant, for comparison purposes, to analyze and show them all (see below).

PCA. The time spent by both residents and intruders performing each scored behavior during the second encounter was subjected to a PCA procedure. Original values, including 146 units representing data of each individual rat containing nine variables (ANO, HEAD, BODY, FOLL, DOM, AGGR, REAR, GROOM, and ENV), were centered and standardized, providing a correlation matrix for the eigen analysis. The respective samples were related to the group's treatment (time duration of the first encounter), the social role (resident or intruder), familiarity with the conspecific during the second encounter (familiar or different) and, preliminarily, four time bins. After this preliminary analysis, only scores relative to the first time bin were included in the PCA due to an increase in dispersion and loss of signal detected through the reduction of the variance explained by the analysis procedure (see below). A square root transformation was used to correct for an implicit quadratic factor related to the properties of the measurement scale, and to homogenize and reduce the dispersion of the original matrix d. The results were represented by the average of each group's scores on a Euclidian biplot. PCA scores were also analyzed by ANOVA.

A preliminary PCA including the four time bins revealed a high colinearity among the responses, possibly due to the fact that they were taken sequentially. Thus, in order to minimize the impact of this factor on the general analysis, only the first time bin scores (measured from zero to 150 s of the second encounter) were included thereafter in the PCA.

Correlations between the loading factors generated by processing individual scores were revealed by the PCA and were interpreted following the guidelines proposed by Cohen (19); i.e., 1) positive 1.00 to 0.50 values and negative

Table 1. Description of the social and non-social behaviors performed by both the resident and the intruder rats.

	Code	Description
Social behaviors		
Sniffing the anogenital region	ANO	The animal's nose is 1 cm or less from the conspecific's anogenital area; the conspecific may be held during sniffing.
Sniffing the head	HEAD	The animal's nose is 1 cm or less from the conspecific's head; the conspecific may be held during sniffing.
Sniffing the body	BODY	The animal's nose is 1 cm or less from the conspecific's body with exception of the anogenital and head areas; the conspecific may be held during sniffing.
Following the conspecific	FOLL	The animal walks towards the conspecific, trying to approach it.
Dominance behavior	DOM	The animal holds the conspecific with the latter's back or ventral part on the floor.
Aggression	AGGR	The animal stands on its hindlegs facing the conspecific and hits the latter with its forepaws.
Non-social behaviors		
Sniffing the environment	ENV	The animal sniffs the cage walls standing on two or four paws (in the latter case, touching the cage walls with its forepaws) or sniffs the bedding.
Self-grooming	GROOM	The animal performs movements such as wiping, licking, and scratching its own head and body fur.
Rearing	REAR	The animal stands on its hindlegs without touching the cage walls and performs up and down body movements, usually sniffing.

-1.00 to -0.50 values were considered to be large correlations, 2) positive 0.49 to 0.30 and negative -0.49 to -0.30 values were considered to be medium correlations, and 3) positive 0.29 to 0.10 or negative -0.29 to -0.10 values were considered to be small correlations.

Since three principal components explained about 66.3% of the data variance, they were further analyzed using a general linear model (GLM) to test the hypothesis that the segregation of all variables applied.

The analyses design included "familiarity", "social role" and "first encounter duration" as the between-subject factors and each component axis as the within-subject factors. The *post hoc* Tukey HSD test was used when applicable. Statistical procedures were run using the Statistical Package for the Social Sciences (SPSS) and the MultiVariate Statistical Package (MVSP); graphs were prepared using the Sigma Plot Package.

Analysis of variance (ANOVA)

The times spent by the rats performing each individually scored behavior and the total time spent performing social and non-social behaviors and their subcategories (social investigatory and social agonistic behaviors) were compared using traditional repeated measures ANOVA. Between-subject factors included 1) "social role" (intruder versus resident), 2) "familiarity" with the conspecific (familiar versus different), and 3) "first encounter duration" (0.083 h (5 min), 0.5 h (30 min), 2, 24, and 168 h (7 days)) and the within-subject factor included 4) "time bins". Separate ANOVAs were run for each individual score and for the total times exhibiting social, non-social, social investigatory, and social agonistic behaviors. The Tukey HSD test was used for *post hoc* comparisons. The level of significance was set at $P < 0.05$. The statistical procedures were carried out with the Statistica software (StatSoft, USA).

Results

Figure 1 shows the total time spent by both resident and intruder rats performing social (Figure 1A,D), social investigatory (Figure 1B,E) and non-social behaviors (Figure 1C,F) during the second encounter. Table 2 shows the percent time of each behavior scored relative to the total time of its corresponding (social and non-social) category. Figure 2 represents the PCA axes (see below). Figures 3, 4, and 5 show the time spent by resident and intruder rats exhibiting social investigatory behaviors, social agonistic behaviors and non-social behaviors, respectively, along the four time bins of the second encounter with either the familiar or a different intruder, separated according to the first encounter duration (see ANOVA for these results below).

PCA

The overall variance was explained by the first and second synthetic axes corresponding to 53.8 and 37.6%, respectively.

The variable loadings on the first and second axes allowed exploration of the main sources of interactions for the

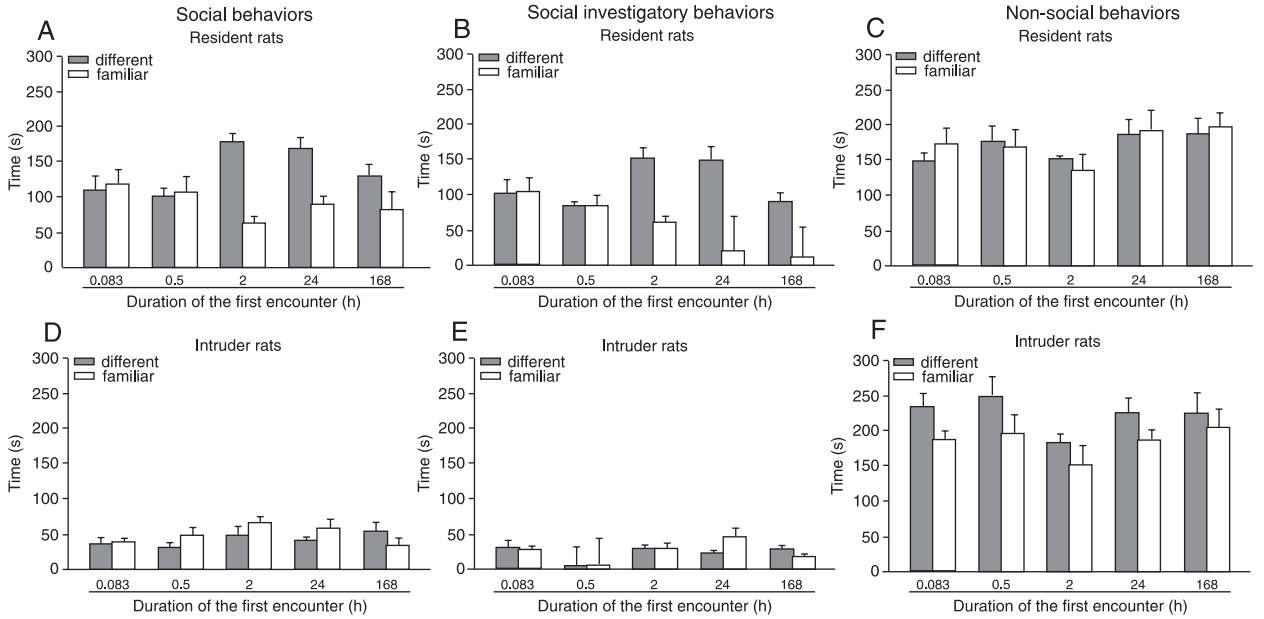

Figure 1. Time spent by resident (top panels) and intruder (bottom panels) rats exhibiting social (A and D), social investigatory (B and E) and non-social behaviors (C and F) during the second encounter with either a familiar (open bars) or a different (filled bars) conspecific, as a function of the duration of the first encounter, which was 0.083, 0.5, 2, 24, or 168 h. Data are reported as means ± SEM.

rats' behavior. Table 3 shows the loading factors for each individual behavior in each of the principal components considered. Table 4 shows the correlation matrix for pairs of behaviors: 1) the large positive correlations involving ANO and BODY, ANO and FOLL, BODY and HEAD, and BODY and FOLL; 2) the large negative correlations between ENV and ANO, ENV and BODY, and ENV and FOLL; 3) the medium positive correlations between ANO and HEAD, HEAD and FOLL, and ENV and REAR, and 4) the medium negative correlations between REAR and ANO, REAR and BODY, and REAR and FOLL.

The variables ANO, HEAD, BODY, and FOLL, in contrast to ENV and REAR, polarized the first component axis and comprised the main source of variance. While ANO, HEAD, BODY, and FOLL exhibited positive correlations with each other, ENV and REAR both exhibited negative correlations with these, representing the main bifurcation in the behavioral patterns. Thus, these behaviors may collectively characterize information gathering. On the positive side of the axis ANO, HEAD, BODY, and FOLL correspond to direct investigation of the conspecific, related to social information gathering. Thus, these individual scores can be

Table 2. Percent of time spent exhibiting each of the behaviors relative to the total time of their social and non-social behaviors.

	% of total time
Social behaviors	
ANO	49.9%
HEAD	3.0%
BODY	17.9%
FOLL	3.1%
DOM	23.8%
AGGR	2.3%
Total	100%
Non-social behaviors	
ENV	76.8%
GROOM	8.9%
REAR	14.3%
Total	100%

For abbreviations, see Table 1.

Table 3. Major contributions to the overall variance (loading factors) on the first, second and third principal components resulting from the principal component analysis applied to the social and non-social behavioral data.

	Components		
	1st	2nd	3rd
Social behaviors			
ANO	0.841*	0.181	-0.241
HEAD	0.591*	0.123	0.569*
BODY	0.819*	-0.082	0.248
FOLL	0.798*	0.283	-0.249
DOM	0.256	-0.704*	0.187
AGGR	-0.029	-0.670*	0.272
Non-social behaviors			
ENV	-0.789*	0.042	0.204
GROOM	-0.023	0.530*	0.696*
REAR	-0.579*	0.307	0.001

Higher loading factors are indicated by asterisks (principal component analysis). For abbreviations, see Table 1.

Table 4. Correlation matrix for pairs of behaviors employed in principal component analysis obtained by the centering and standardization of the original matrix.

Behaviors	HEAD^{+}	BODY^{+}	FOLL^{+}	DOM^{+}	AGGR^{+}	ENV$^{\#}$	GROOM$^{\#}$	REAR$^{\#}$
ANO^{+}	0.352*	0.541*	0.735*	0.030	-0.095	-0.679*	-0.038	-0.388*
HEAD^{+}		0.562*	0.324*	0.132	-0.024	-0.283	0.210	-0.247
BODY^{+}			0.545*	0.280	0.054	-0.547*	0.038	-0.400*
FOLL^{+}				0.003	-0.196	-0.575*	-0.010	-0.324*
DOM^{+}					0.207	-0.174	-0.188	-0.243
AGGR^{+}						-0.036	-0.092	-0.094
ENV$^{\#}$							0.087	0.333*
GROOM$^{\#}$								0.098
REAR$^{\#}$								

The main significant correlations are indicated by asterisks (ANOVA). $^{+}$Social behaviors; $^{\#}$non-social behaviors. For abbreviations, see Table 1.

grouped as characterizing social investigatory behavior. Note that these scores were mainly correlated with the resident rats' behavior (Figure 2, circles, and Figure 1A,B), particularly those exposed to different conspecifics in the second encounter (Figure 2, black circles, and Figure 1A,B, black bars), after being exposed to a conspecific for 2 h or longer (Figure 2, greater black circles, and Figure 1A,B). As a matter of fact, resident rats exposed to longer first encounters (2, 24, and 168 h) exhibited more social investigation towards different intruders compared to that seen towards the familiar intruder (Figure 2, longer distance between the white and black circles of corresponding sizes for larger, but not smaller, circles, and Figure 1A,B). Conversely, resident rats exposed to shorter first encounters (0.083 and 0.5 h) did not exhibit any difference in social investigation towards the familiar or different intruder (Figure 2, shorter distance between the white and black circles of corresponding smaller sizes, and Figure 1A,B). On the negative side of the first principal component axis, ENV and REAR characterized non-social (or environmental) information gathering, being mainly correlated with the intruder rat's behavior (Figure 2, triangles, and Figure 1F).

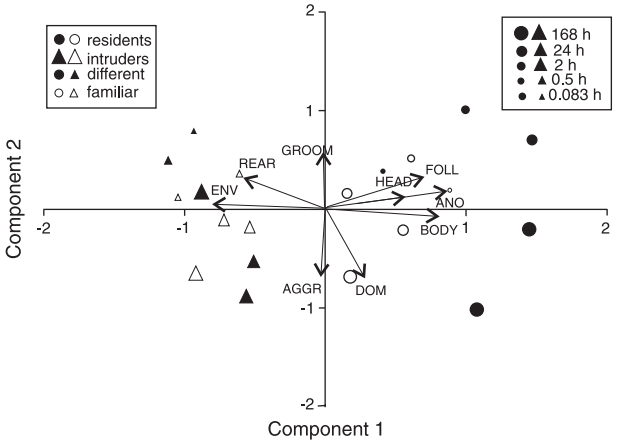

Figure 2. Euclidian biplot of the variables and average group scores on the first and second axes resulting from the principal component analysis applied on the correlation matrix that was obtained by centering and standardizing the original data. The first component, explaining 37.6% of the variance in the matrix, may collectively be characterized as information gathering. It included ANO, BODY, HEAD, and FOLL, which relate to social information gathering, and, in contrast, ENV and REAR, which refer to environmental information gathering. While social investigatory behaviors were mainly generated by resident rats (circles), non-social behaviors were mainly generated by the intruder rats (triangles). The second principal component, explaining 16.1% of the variance in the matrix, seems to be characterized by agonistic-induced self-conflict since it was determined by GROOM opposed by AGGR and DOM. Thus, it seems to relate to social interactions that contribute to the establishment of hierarchical relationships in social groups; while longer first encounters induced greater AGGR and DOM scores (greater circles and triangles), smaller first encounters induced greater GROOM scores (smaller circles and triangles), expressing self-conflict. ANO = anogenital sniffing; BODY = body sniffing; HEAD = head sniffing; FOLL = following; ENV = sniffing the environment; REAR = rearing; GROOM = self-grooming; AGGR = aggression; DOM = dominance.

Figure 2 also shows that the second principal component was mainly determined by GROOM, usually considered to reflect an individual conflict-related behavior, on the positive side of the axis, in contrast to AGGR and DOM, on the negative side of the same axis, related to social interactions that contribute to the establishment of hierarchical relationships in social groups. Thus, the second component of the PCA seems to be characterized by agonistic-induced self-conflict. The AGGR and DOM behaviors are usually included among social behaviors; however, the present analysis revealed that their nature differed from that of social investigatory behaviors (compare Figures 3 and 4). Thus, it seemed plausible to include them in a different subcategory named social agonistic behavior. In general, independent of the social role, there seems to be a segregation; while GROOM behavior was mainly observed in subjects exposed to shorter first encounters (Figure 2, smaller circles and triangles on the positive side of the second component), reflecting greater conflict, social agonistic behaviors were mainly seen in subjects exposed to longer first encounters (Figure 2, larger circles and triangles on the negative side of the second component). These figures suggest that shorter first encounters lead to greater expression of individual conflict-related behaviors during the second encounter, but that longer first encounters lead to greater agonistic-induced behaviors during the second encounter (Figures 2 and 4).

A GLM for PCA scores on the first principal component revealed significant effects for the main factors of 1) "social role" ($F_{1,145} = 10.051$, $P < 0.0001$) and 2) "familiarity" ($F_{1,145} = 3.019$, $P < 0.005$); in addition, it revealed significant interaction effects for 3) "familiarity" and "social role" ($F_{1,145} = 12.652$, $P < 0.001$), 4) "familiarity" and "first encounter duration" ($F_{4,145} = 4.012$, $P < 0.005$), and 5) "familiarity", "social role" and "first encounter duration" ($F_{4,145} = 3.263$, $P < 0.05$). Thus, the increase in the first encounter duration rendered resident rats capable of recognizing the familiar intruder and discriminating it from the different intruder, in spite of a 24-h time interval between the first and second encounters.

A similar GLM analysis for critical PCA scores of the second principal component revealed significant effects of the "first encounter duration" ($F_{4,145} = 10.544$, $P < 0.0001$). As a matter of fact, while shorter first encounters resulted in both resident and intruder rats exhibiting more GROOM behavior, longer first encounters resulted in them exhibiting more AGGR and DOM behaviors (Figure 2, smaller circles and triangles on the positive side of the second component, and larger circles and triangles on the negative side of it).

ANOVA

Table 5 shows the P values of the ANOVA involving each scored social (Figure 1A,D) and non-social (Figures 1C,F and 5) behavior and the subcategories of social

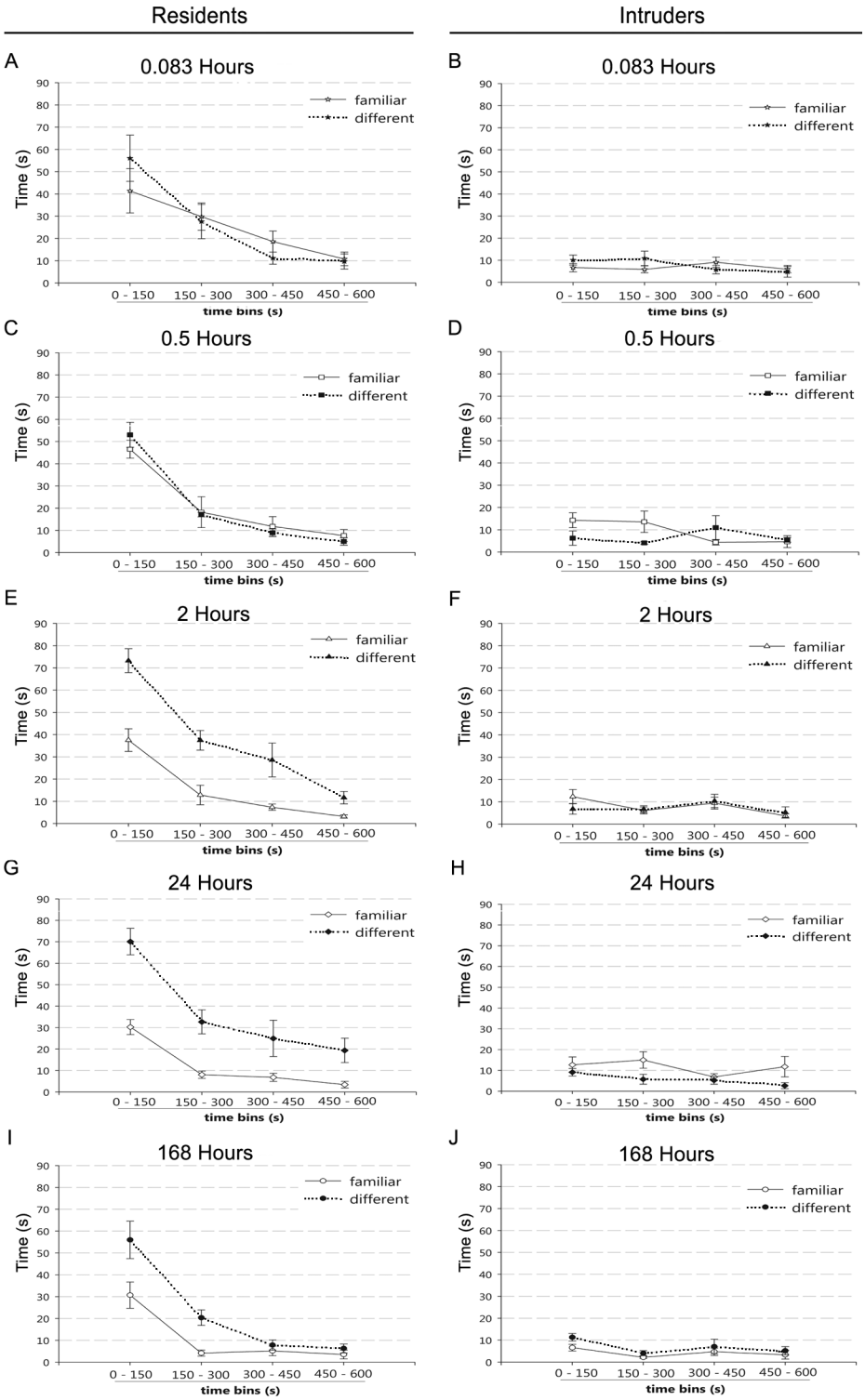

Figure 3. Time spent by resident (left panels) and intruder (right panels) rats exhibiting social investigatory behaviors during the second encounter with either a familiar (continuous line) or a different (dotted line) conspecific, as a function of four successive time bins, after a first encounter with a duration of 0.083 (A and B), 0.5 (C and D), 2 (E and F), 24 (G and H), or 168 (I and J) h. Data are reported as means ± SEM.

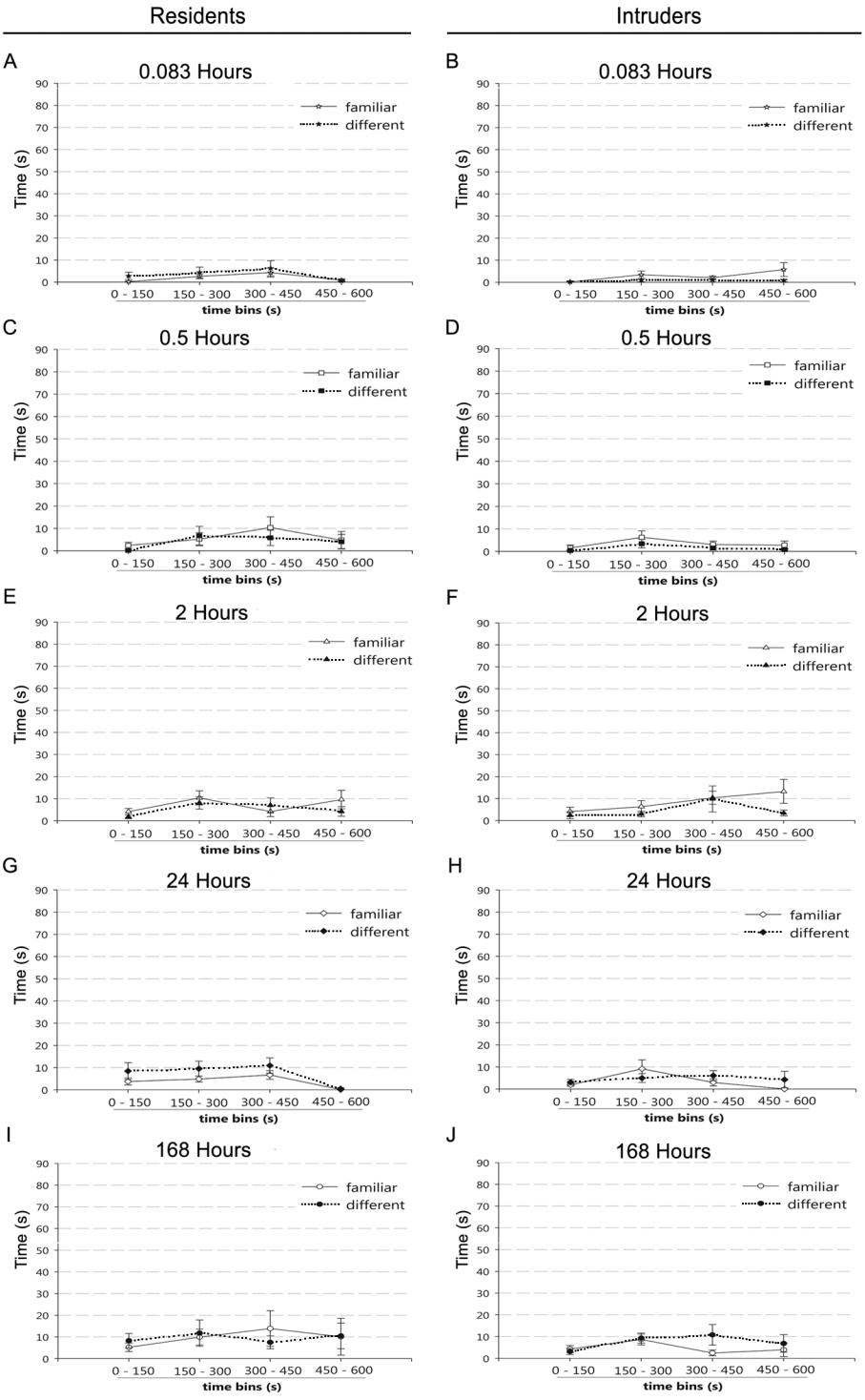

Figure 4. Time spent by resident (left panels) and intruder (right panels) rats exhibiting social agonistic behaviors during the second encounter with either a familiar (continuous line) or a different (dotted line) conspecific, as a function of four successive time bins, after a first encounter with a duration of 0.083 (A and B), 0.5 (C and D), 2 (E and F), 24 (G and H), or 168 (I and J) h. Data are reported as means ± SEM.

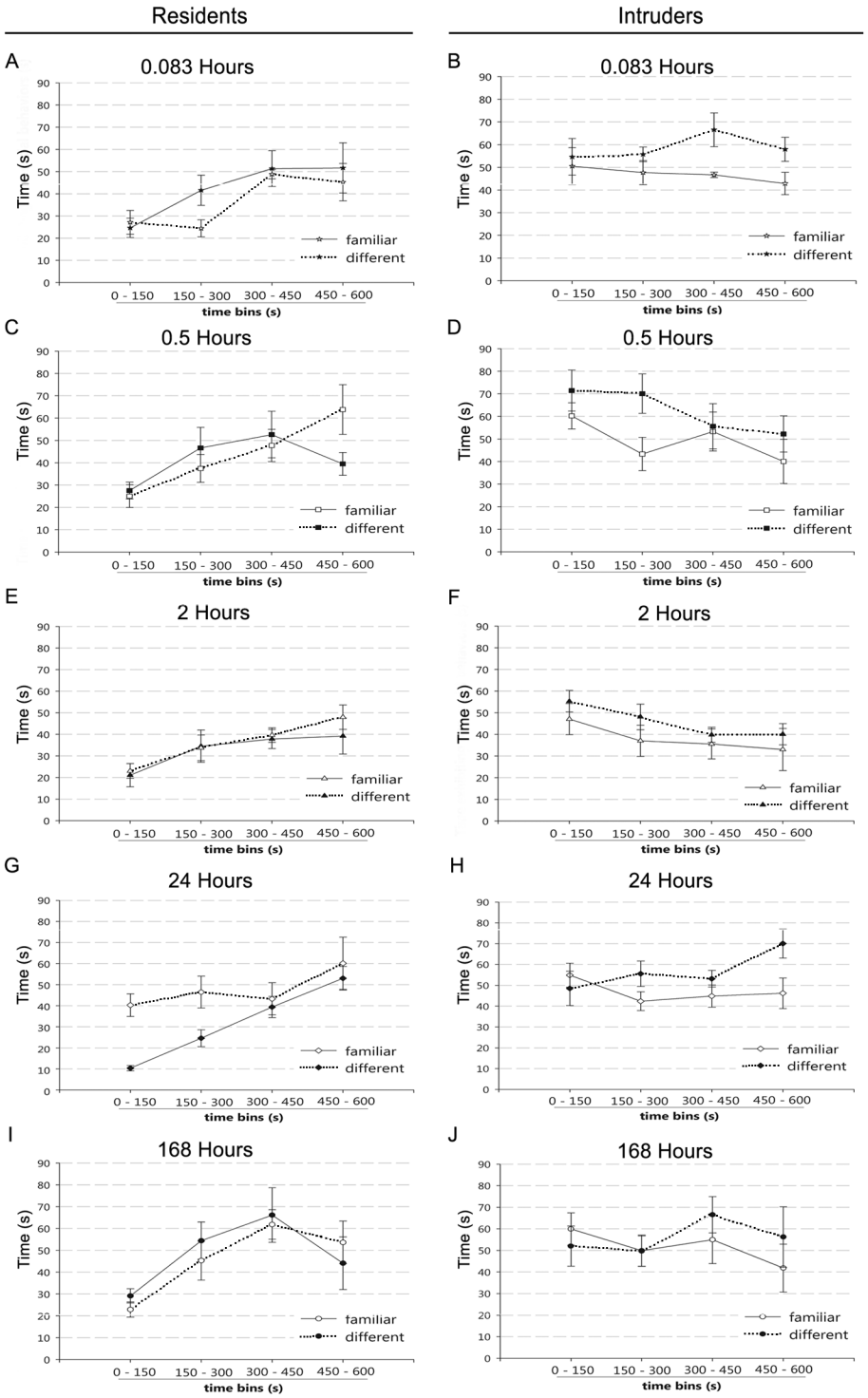

Figure 5. Time spent by resident (left panels) and intruder (right panels) rats exhibiting non-social behaviors during the second encounter with either a familiar (continuous line) or a different (dotted line) conspecific, as a function of four successive time bins, after a first encounter with a duration of 0.083 (A and B), 0.5 (C and D), 2 (E and F), 24 (G and H), or 168 (I and J) h. Data are reported as means ± SEM.

investigatory (Figures 1B,E and 3) and social agonistic (Figure 4) behaviors.

As expected, these results essentially confirm the major outcomes of the PCA. Together, they indicate that the resident rats exhibited more social behaviors, particularly social investigatory behaviors, relative to the intruder rats (compare Figure 1A,D, 1B,E, and the left and right panels of Figure 3), especially when the second encounter involved a different intruder and when the first encounter lasted 2 h or longer (Figures 1A,B and 3E,G,I). In addition, note that these social investigatory behaviors were greater at the beginning of the second encounter (Figure 3, left panels), decreasing along time bins (Figure 3E,G,I). Furthermore, very long first encounters (168 h) accelerated this decrease of social investigation towards a different intruder (compare Figure 3E,G,I, during second, third and fourth time bins).

Even though, as expected, the behavioral outcomes reflected by social behavior (Figure 1A) and the subcategory social investigatory behaviors (Figure 1B) were similar, while social investigatory behaviors towards the familiar intruder consistently decreased as a function of the increase in duration of the first encounter (Figure 1B, open bars), total social behaviors did not decrease (Figure 1A, open bars). This effect was related to the significant increase of the subcategory of social agonistic behavior for both resident and intruder rats as the duration of the first encounter increased (Figure 4). As a consequence, though resident rats exposed to an intruder for 168 h did exhibit a significant difference in the time spent exhibiting social investigatory behaviors towards a different intruder as compared to the familiar intruder ($P < 0.05$, Tukey test), this difference was not seen for the time spent exhibiting total social behaviors ($P > 0.05$, Tukey test).

The intruder adult rats exhibited greater non-social behavioral scores compared to resident rats (Figure 1C,F). Interestingly, this effect was stronger when the intruder rats were exposed to a different resident rat compared to exposure to the familiar resident rat (Figures 1F and 5, right panels). Since 76.8% of the non-social behaviors correspond to sniffing the environment (Table 2), these results suggest that the intruder rats are also capable of recognizing the conspecific they had been previously exposed to by sniffing the cage walls and bedding where the resident rat was maintained, leaving its olfactory signature scent in it (see below).

A gradual increase of non-social behaviors along time bins was observed in resident rats, in general, reaching the highest level in the last time bin (450-600 s; Figure 5, left panels). This result might reflect the time course of priorities of the resident rat when exposed to an intruder rat; that is, the resident rat starts investigating the intruder at the beginning of the encounter and then investigates the environment. This interpretation also applies to the occurrence of the increased expression of non-social behaviors by resident rats at the beginning of the exposure to a familiar conspecific (e.g., Figure 5G). Similar effects were not seen in the intruder rats (Figure 5) that spent longer times exhibiting non-social behaviors during the second encounter (Figure 5, right panels), particularly when exposed to a different resident rat as compared to exposure to the familiar resident rat (Figures 1F and 5, right panels).

Table 5. P values resulting from traditional ANOVAs involving each behavior scored as a function of familiarity, social role, 1st encounter duration, and time bins, as well as their interactions for each scored behavior including total time exhibiting social (SOC), and non-social (NSOC) behaviors, as well as the sub-categories of social investigatory (SOCinv) and social agonistic behaviors (SOCago).

	ANO	BODY	HEAD	FOLL	DOM	AGGR	ENV	REAR	GROOM	SOC	SOCinv	SOCago	NSOC
Familiarity	0.005*	0.05*	0.005*	0.001*	0.60	0.74	0.05*	0.05*	0.76	0.005*	0.0005*	0.58	0.29
Social role	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.08	0.28	<0.0001*	0.01*	0.075	<0.0001*	<0.0001*	0.12	<0.0001*
1st encounter duration	0.05*	0.62	0.35	0.001*	0.01*	0.01*	0.11	0.0005*	0.05*	0.17	0.05*	0.005*	0.28
Time bins	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.16	0.01*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Familiarity x social role	<0.0005*	0.05*	0.05*	0.005*	0.82	0.57	0.094	0.005*	0.89	<0.0001*	<0.0001*	0.77	0.01*
Familiarity x 1st encounter duration	0.11	0.05*	0.12	0.73	0.52	0.05*	0.94	0.05*	0.05*	0.01*	0.01*	0.41	0.70
Social role x 1st encounter duration	0.25	0.22	0.38	0.01*	0.55	0.96	0.77	0.89	0.13	0.94	0.31	0.58	0.86
Familiarity x time bins	0.37	0.005*	0.21	0.083	0.73	0.46	0.0005*	0.44	0.29	0.05*	0.05*	0.74	0.01*
Social role x time bins	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.42	0.63	<0.0001*	<0.0001*	0.23	<0.0001*	<0.0001*	0.54	<0.0001*
1st encounter duration x time bins	0.46	0.0005*	0.68	0.005*	0.45	0.05*	0.005*	0.52	0.55	0.86	0.30	0.42	0.005*
Familiarity x social role x 1st encounter duration	0.01*	0.01*	0.001*	0.55	0.98	0.70	0.54	0.81	0.41	0.005*	0.05*	0.97	0.70
Familiarity x social role x time bins	0.05*	0.20	0.27	0.05*	0.15	0.11	0.56	0.69	0.58	0.005*	0.005*	0.26	0.18
Familiarity x 1st encounter duration x time bins	0.10	0.28	0.76	0.09	0.78	0.64	0.21	0.11	0.87	0.13	0.05*	0.84	0.41
Social role x 1st encounter duration x time bins	0.42	0.01*	0.96	0.05*	0.53	0.74	0.40	0.40	0.13	0.49	0.76	0.53	0.78
Familiarity x social role x 1st encounter duration x time bins	0.07	0.29	0.52	0.05*	0.29	0.47	0.83	0.90	0.99	0.005*	0.005*	0.42	0.71

Discussion

To our knowledge, the results of the present study show for the first time that male rats exhibit social recognition memory that lasts at least 24 h. This effect was revealed by a significantly longer social investigation by the resident rats exposed to a different adult intruder conspecific as compared to exposure to the adult familiar intruder conspecific (Figure 1A,B).

In addition, the results of the present study also showed that no social recognition memory was seen in the second encounter occurring 24 h after a first encounter lasting 5 or 30 min (Figure 1A,B). This particular observation agrees with reports that a resident rat's social recognition memory of a juvenile intruder conspecific does not last more than 60 min when the duration of the first encounter is 30 min or less (4,6-12,20).

Duration of social recognition memory

Prior attempts to enhance retention of the social recognition memory in rats involved manipulation of different aspects of the intruder-resident paradigm (6,7,18).

Dantzer et al. (7) gathered evidence showing that adult rats recognized a juvenile after a single 5-min exposure to it when testing occurred 30 min, but not 2 h, after the first encounter. This social memory was enhanced after another 5-min exposure to the familiar juvenile. This result suggested that an increase in the duration of the first encounter could prolong social memory, as we show in the present study.

Sekiguchi et al. (6) found no evidence that resident rats could recognize a juvenile met for a total of 2 h (divided into multiple shorter sessions) when a 24-h interval between encounter sessions was used. However, Sekiguchi et al. (6) exposed the familiar juvenile intruder six times per day for four days, while in the present study an adult intruder was presented in a single 2-h session. This continuous 2-h exposure to the conspecific intruder may have helped the resident rat to gather more consistent information for later memory recognition. In addition, while Sekiguchi et al. (6) used a juvenile intruder, in the present study we used an adult intruder. Since an adult conspecific may represent a potential competitor for the territory of the resident animal, remembering a previous encounter with it may represent a more meaningful experience compared to a previous encounter with the juvenile intruder, thus improving memory. Furthermore, the resident rats in the experiment of Sekiguchi et al. (6) were 1.5 years old while the ones used in the present study were 3 months old. It is well known that the memory of young adult rats is better than that of old adult rats (21,22).

The results of the present study showing that long-term coexistence increases the duration of social recognition memory do not conflict with Burman and Mendl's (14) who reported that female juveniles discriminate the odor from a former female cage-mate up to 48 h after separation. In addition, the present research extended their study showing that 2 h of continuous exposure to a male con-specific adult rat was enough to generate a long-term social recognition memory, thus providing a feasible model for investigating the processes underlying this type of long-term memory.

Critical categories of social behaviors

In agreement with the literature in the area (6,7,11,17,18) we included ANO, HEAD, BODY, FOLL, DOM, and AGGR scores in the category of social behaviors. However, the PCA involving individually scored behaviors revealed that they are coherently segregated into two main independent principal components (Figure 2). Thus, in addition to the collective "social behavior" category, two relevant subcategories were defined, social investigatory behaviors (including ANO, HEAD, BODY, and FOLL) and social agonistic behavior (including DOM and AGGR).

The present study revealed both 1) a substantial decrease in social investigatory behaviors towards the familiar intruder with increasing duration of the first encounter (Figure 1B, open bars) and 2) an increase of social investigatory behaviors towards a different intruder when the first encounter lasted 2, 24 and, to a lesser extent, 168 h (Figure 1B, filled bars). This demonstrates that rats do exhibited a 24-h long social recognition memory when the first encounter lasted 2, 24, and 168 h.

Interestingly, there was no significant decrease in the time spent by the resident rats performing (total) social behaviors when the residents were exposed to the intruders for 168 h in the first encounter (Figure 1A, open bar). This occurred because the time spent by the resident rats exhibiting social agonistic behaviors significantly increased with increasing duration of the first encounter (Figure 4), compensating for the decrease seen in the time spent exhibiting social investigatory behaviors. The importance of this effect should not be underestimated; the use of either the category of social behaviors, as employed by most of studies in the area, or the subcategory of social investigatory behavior, as also employed in this study, could lead to different conclusions. As shown above, resident rats whose first encounters lasted 168 h did exhibit significant differences in the time spent exhibiting the subcategory of social investigatory behaviors towards a different intruder compared to that seen towards a familiar intruder and thus could be considered to exhibit a 24-h duration social recognition memory. However, this comparison was not statistically significant for the category of social behaviors. This fact would then lead to the conclusion that rats do not exhibit such a social recognition memory. Since many studies using the intruder-resident paradigm adopt a general "social behavior" category (1,6,9,11,17), which includes a diversity of behaviors that may, in fact, be characterized as social but not necessarily related to social investigation (e.g., mild aggression, rolling/standing over the juvenile and/or pushing it away, dominance, social grooming), their

potential to detect social recognition memory may have been limited, as exemplified in the present study by the different statistical results when comparing social behaviors and social investigatory behaviors. In fact, some prior studies excluded aggressive behavior from the "social behavior" category (4,7,10,18).

Most of the social investigation by the resident rats occurred in the first time bin and then decreased along time bins (Figure 3). The time spent by the resident rats exhibiting social investigatory behaviors towards the familiar and the different intruders indicates that longer first encounters accelerate the rate of reduction of social investigation along time bins in spite of the lack of social recognition. It is as if there was a transfer of habituation of the social investigatory activity from the first to the second encounters and this habituation was sensitive to the duration of the first encounter.

This effect also occurred for the resident rats whose first encounters lasted 2 (Figure 3E), 24 (Figure 3G), and 168 h (Figure 3I); in fact, the stronger reduction of social investigatory behaviors towards the familiar intruders associated with longer first encounters supports this interpretation. However, since first encounters lasting 2 h or longer allow gathering enough information for social recognition memory, the resulting increased social investigation towards a different intruder would have overcome this habituation effect, masking it. Accordingly, resident rats whose first encounter lasted 168 h exhibited lesser social investigation and greater transfer of habituation to the second encounter (Figure 3I), which contributed to the reduction of time spent exhibiting social investigatory behaviors.

Social recognition memory in the intruder rats?

The adult intruder rats involved in the present study did not present any significant differences in the time performing social investigatory behaviors towards a familiar or a different intruder (Figure 1E). Even though these data could be interpreted as reflecting a lack of social recognition memory by the intruder rats, one has to be cautious about this interpretation (see below).

Thor and Holloway (18) reported that juvenile intruders exhibit social recognition memory only when the time interval between the first and the second encounter is less than 4 min. These investigators concluded that social recognition memory is shorter in younger rats than in adult rats. It would be tempting to ascribe this apparent lack of social recognition memory by our intruder rats to the 24-h time interval between the first and the second encounter. However, the intruder rats involved in the present experiment were as old as the resident rats, permitting us to rule out this possibility.

Procedural differences between the treatments of resident and intruder rats may have provided a more disturbing experimental condition for the intruder rats compared to the resident rats, thus interfering with their social recognition memory (11). Favoring this interpretation (Moura PJ, Venkitaramani DV, Tashev R, Lombrosso PL, Xavier GF, unpublished observations) showed that transportation of resident rats from the experimental room to the animal facilities 0.5, but not 6 h, after the first encounter strongly interfered with social recognition memory tested in a second encounter 24 h later.

It was noticeable that exposure of the so-called "resident rats" to the testing chamber for only 20 min before the beginning of the first encounter was enough to determine that they would assume a behavioral pattern that characterized them as "residents", similar to mice (16). First, they had the opportunity to urinate in the clean testing chamber and in the novel bedding, thus giving to this environment its individual signature scent (23), which could then modulate the reaction of both residents and intruders. Second, because they had the opportunity to explore the chamber before the intruder, they concentrated their investigation on the intruder, gathering relatively more social information. The relative contribution of each of these factors to the amount of social and non-social investigation in the second encounter is not clear.

Intruder rats exhibited longer times of non-social behaviors (Figure 1F,C, respectively, and Figures 2 and 5). This effect was stronger when the intruder rats were exposed to a different resident rat than to the familiar resident rat (Figures 1F and 4). It could be thought that this increased environmental investigation reflects a reaction to odors of different conspecifics present in the testing chamber (see Ref. 23), when the intruder is different. Intruder rats exposed to familiar residents were introduced into a chamber containing only the odors of the resident rats, in addition to their own odor possibly left on the chamber walls and bedding during the first encounter. This could help to understand why non-social investigation was greater for intruder rats exposed to different resident rats. The present data do not allow further evaluation of these hypotheses.

Interestingly, resident rats exhibited a gradual increase of the time spent exhibiting non-social behaviors along time bins (Figure 5, left panels). This may reflect the investigative priorities of the resident rat when exposed to an intruder rat, that is, after investigating the intruder at the beginning of the encounter, the resident rat investigates the environment in order to evaluate to what extent the intruder introduced any changes in "his territory". Similar effects were not seen in the intruder rats, who spent longer times exhibiting non-social behaviors along the complete second encounter (Figure 5, right panels).

Critical scores in the intruder-resident paradigm

The PCA included in the present study allowed the detection of behavioral patterns indicating the occurrence of two relevant and independent sub-categories of social behaviors, one of them named "social investigatory behaviors", related to social information gathering, and the other

named “social agonistic behaviors”, related to the establishment of social hierarchies. Among the social investigatory behaviors, anogenital and body sniffing corresponded to about 50 and 18%, respectively, of the total social behaviors (Table 2). On the other hand, among the social agonistic behavior, dominance corresponded to about 24% of the total social behaviors. While social investigatory behaviors were exhibited mainly by resident rats exposed to different intruders, social agonistic behaviors were exhibited mainly by both resident and intruder rats exposed to the conspecific for a longer period of time.

Previous studies have reported the critical importance of anogenital investigation for social recognition memory. For instance, Ferguson et al. (3) reported that male rodents not only closely sniff the anogenital region but also lick it. These investigators emphasized the fact that male rodents usually do not sniff conspecifics at a distance, which would imply that the male is utilizing pheromonal, nonvolatile odorants to recognize the conspecific (24,25). Apparently, the rat’s urine contains chemical compounds important for social recognition; supposedly, when the rat performs self-grooming it spreads olfactory cues along the body and head (8), rendering itself recognizable to conspecifics. This would explain why sniffing the anogenital region and the body strongly contributed to social investigatory behaviors in the present study.

While sniffing the environment was responsible for about 76.8% of the non-social behaviors, rearing was responsible for about 14% of them. Most of the environmental sniffing was directed towards the cage walls and bedding. It is known that dirt bedding has putative volatile odorants and nonvolatile compounds, including urinary proteins that have been suggested to be involved in intra-specific communication (26,27). Thus, even though sniffing the environment could also provide social information, this seemed to have contributed to social recognition only in a limited fashion. PCA revealed that ENV was positively correlated with REAR and negatively correlated with ANO, BODY and FOLL, suggesting that the subjects exhibiting ENV were only gathering environmental information and not social information. However, the present data do not allow formulation of a clearer picture about this issue and more studies would be required to evaluate these possibilities.

The results of the present study showed that male rats exhibit long-term social recognition memory that lasts at least 24 h after a single 2 h or longer exposure to the adult male conspecific. In addition, results showed that the subcategory of social investigatory behaviors corresponded to a more appropriate index of social recognition memory than the one usually adopted in other laboratories, that also include social agonistic behaviors. This demonstration of long-term social recognition memory in rats opens several possibilities of using this behavioral paradigm for studies involving identification of cellular and molecular mechanisms involved in this type of memory. In addition, it also provides a new behavioral model for studying manipulations that may interfere with memory consolidation.

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References

1. Popik P, Wolterink G, De Brabander H, van Ree JM. Neuropeptides related to [Arg8]vasopressin facilitates social recognition in rats. *Physiol Behav* 1991; 49: 1031-1035.
2. Carr WJ, Yee L, Gable D, Marasco E. Olfactory recognition of conspecifics by domestic Norway rats. *J Comp Physiol Psychol* 1976; 90: 821-828.
3. Ferguson JN, Young LJ, Insel TR. The neuroendocrine basis of social recognition. *Front Neuroendocrinol* 2002; 23: 200-224.
4. Gheusi G, Bluthe RM, Goodall G, Dantzer R. Ethological study of the effects of tetrahydroaminoacridine (THA) on social recognition in rats. *Psychopharmacology* 1994; 114: 644-650.
5. Young LJ. The neurobiology of social recognition, approach, and avoidance. *Biol Psychiatry* 2002; 51: 18-26.
6. Sekiguchi R, Wolterink G, van Ree JM. Short duration of retroactive facilitation of social recognition in rats. *Physiol Behav* 1991; 50: 1253-1256.
7. Dantzer R, Bluthe RM, Koob GF, Le Moal M. Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology* 1987; 91: 363-368.
8. Popik P, van Ree JM. Neurohypophyseal peptides and social recognition in rats. *Prog Brain Res* 1998; 119: 415-436.
9. Popik P, Vetulani J, Bisaga A, van Ree JM. Recognition cue in the rat’s social memory paradigm. *J Basic Clin Physiol Pharmacol* 1991; 2: 315-327.
10. Prediger RD, Batista LC, Miyoshi E, Takahashi RN. Facilitation of short-term social memory by ethanol in rats is mediated by dopaminergic receptors. *Behav Brain Res* 2004; 153: 149-157.
11. Burman OH, Mendl M. Short-term social memory in the laboratory rat: its susceptibility to disturbance. *Appl Anim Behav Sci* 2000; 67: 241-254.
12. Prediger RD, Takahashi RN. Ethanol improves short-term social memory in rats. Involvement of opioid and muscarinic receptors. *Eur J Pharmacol* 2003; 462: 115-123.
13. Marin JC, Moura PJ, Cysneiros RM, Colugnati DB, Cavalheiro EA, Scorza FA, et al. Temporal lobe epilepsy and social behavior: an animal model for autism? *Epilepsy Behav* 2008; 13: 43-46.
14. Burman O, Mendl M. Long-term social memory in the laboratory rat (*Rattus norvegicus*). *Anim Welf* 2006; 15: 379-382.
15. Moura PJ, Gimenes-Junior JA, Valentinuzzi VS, Xavier GF. Circadian phase and intertrial interval interfere with social

recognition memory. *Physiol Behav* 2009; 96: 51-56.
16. Kogan JH, Frankland PW, Silva AJ. Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus* 2000; 10: 47-56.
17. Burman OH, Mendl M. The effects of environmental context on laboratory rat social recognition. *Anim Behav* 1999; 58: 629-634.
18. Thor DH, Holloway WR. Social memory of the male laboratory rat. *J Comp Physiol Psychol* 1982; 96: 1000-1006.
19. Cohen J. *Statistical power analysis for the behavioral sciences*. Hillsdale: Erlbaum; 1988.
20. Engelmann M, Landgraf R. Microdialysis administration of vasopressin into the septum improves social recognition in Brattleboro rats. *Physiol Behav* 1994; 55: 145-149.
21. Erickson CA, Barnes CA. The neurobiology of memory changes in normal aging. *Exp Gerontol* 2003; 38: 61-69.
22. Roman FS, Alescio-Lautier B, Soumireu-Mourat B. Age-related learning and memory deficits in odor-reward association in rats. *Neurobiol Aging* 1996; 17: 31-40.
23. Sawyer TF, Hengehold AK, Perez WA. Chemosensory and hormonal mediation of social memory in male rats. *Behav Neurosci* 1984; 98: 908-913.
24. Halpern M. The organization and function of the vomeronasal system. *Annu Rev Neurosci* 1987; 10: 325-362.
25. Wysocki CJ, Wellington JL, Beauchamp GK. Access of urinary nonvolatiles to the mammalian vomeronasal organ. *Science* 1980; 207: 781-783.
26. Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DH, et al. Individual recognition in mice mediated by major urinary proteins. *Nature* 2001; 414: 631-634.
27. Jemiolo B, Alberts J, Sochinski-Wiggins S, Harvey S, Novotny M. Behavioral and endocrine responses of female mice to synthetic analogues of volatile compounds in male urine. *Anim Behav* 1985; 33: 1114-1118.