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Research report

# Emotional changes related to age in rats—a behavioral analysis

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**Abstract**

The present study investigated age-related differences in the emotional behavior of rats using factor analysis to identify motivational factors influencing spontaneous behavior in open field with illuminated center (OF), plus maze (EPM) and social interactions test. Animals of the same strain, bred under the same conditions, formed two experimental groups: young adults (YA, $N = 20$) tested at the age of 4 months and old rats (OA, $N = 16$) tested at the age of 24 months. The computer video based tracking system EthoVision was used for automated acquisition and analysis of data. The results of each test were analyzed separately for YA and OA by factor analysis. Two main independent factors emerged from the analysis of OF measures—factor 1, which appeared to reflect motor activity, and factor 2, reflecting anxiety. The measures best reflecting motor activity (distance moved in the peripheral zone) and anxiety (time spent in central zone) decreased significantly with age. Factor analysis for EPM measures revealed, in both groups, three independent factors. In YA, factor 1 reflected motor activity, factor 2—anxiety, in OA measures of anxiety loaded on factor 1, measures of activity on factor 2. Factor 3 in both groups appeared to represent a decision making process. The number of entries to the closed arms declined significantly in OA, showing an age related decrease of motor activity. Also, the ratio of open arms entries in relation to the total number of entries decreased in OA, indicating a higher anxiety level. Three independent factors emerged from the analysis of social interaction measures. The pattern of factor loading was different in young and old animals, although the number and time of social interactions did not show age-related differences. In addition to a decrease of motor activity we conclude that old rats also differ from young animals in emotional and social behavior. 

*Keywords:* Emotions; Aged rats; Factor analysis; PCA; Open field; Elevated plus maze

## 1. Introduction

Changes in emotional behavior represent one of the important aspects of aging in humans and are a consistent focus of the gerontological literature [16,20,25,38]. They are often considered as secondary to personal experience, socioeconomic conditions and lifestyle. However, the differences between young and old animals in emotional reactivity have been noted in ethological observations as well. Relatively few systematic studies have been devoted to the evaluation of emotional behavior in old animals. It has been found that age-related changes occur in emotional reactivity to a novel environment, as assessed by performance of rats in the open field and hole box tests [4,24,31]. Also, in a plus-maze task which is based on the natural aversion of rodents for open space conflicting with the drive to explore a new environment, significant changes indicating the increased level of anxiety were observed in old mice [23] and rats [10]. It might be supposed that hyperemotionality and/or increased anxiety affects the social interactions of old animals. Some authors [6,37] have reported decrease of aggressiveness toward conspecifics in old rats and mice compared with young adults, while Blanchard et al. [2,3], on the basis of longitudinal observations, did not find age-related changes in aggressive behavior during social encounters.

It is well known that aging is a multifactorial process [4,11–13,22,23,27,31]. A variety of neurobehavioral functions decline at different rates with age in animals as well as in humans. While some deficits occurring with age are interrelated, some others appear to arise

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independently from one another. For instance, it has already been found that impairment of learning and memory in aged rats can be dissociated from changes in motor and emotional behavior [31]. Blokland and Raaijmakers [4] reported a lack of correlation between cognitive and noncognitive parameters of behavior in aged rats. Gage et al. [12] demonstrated no relationship between the severity of sensorimotor deficits and the degree of learning impairment among old rats.

The present experiment was intended to study the spontaneous behavior of old rats in comparison to young adults in a set of standard laboratory tests designed to examine the level of anxiety and social interactions. Spontaneous behavior in the open field, plus maze and during social encounters includes emotional as well as motor components. Most authors have described an age-related decline in horizontal motor activity in rodents [12,18,23,35], however, there are also studies that have not found such a result [17,19,30]. In some cases, a decrease in habituation to motor activity was observed with aging [9].

The aim of our study was to differentiate possible motivational factors influencing spontaneous behavior in various tasks with the use of factor analysis and therefore to further explore the question of age-related changes in emotional behavior. Factor analysis is considered a particularly beneficial statistical tool for the interpretation of behavioral data [7,33,34]. In our experiment, factor analysis was applied for three principal reasons: to regard the individual differences between subjects—it is well known that marked variation in behavioral impairments is seen between individuals of the same aged rat population [12,26], to identify the relationship between specific test indices and factors such as motor activity, anxiety and social or/and aggressive tendencies, and finally, to assess the applicability of commonly used behavioral tests to the investigation of aged animals.

## 2. Materials and methods

### 2.1. Animals

The experiments were performed on 36 male Wistar rats: 20 young (YA) i.e. 4 months of age, weighing 370–450 g and 16 old (OA) i.e. 24 months of age, weighing 470–630 g. All animals were bred in the licensed animal husbandry of the Institute of Occupational Medicine in Lodz, Poland and kept in collective plastic cages with food and water ad lib under a 12 h:12 h light/dark schedule. The behavioral recordings took place between 10:00 and 16:00 h.

The project was approved by the Local Ethics Committee at the Nencki Institute.

### 2.2. Sensorimotor tasks

Before the proper experiment, the rats were subjected to a series of sensorimotor tasks designed to assess muscle strength and coordination, equilibrium and orienting reactions [29]. Each test (maximum 60 s) was given once a day for 3 consecutive days.

- Turning in an alley. The rat was placed facing the back wall of an alley (6 cm wide, 30 cm high). The time of turning around to face the open end of the alley was recorded.
- Turning on an inclined screen. The rat was placed on the center of a horizontal screen covered by rough fabrics (35 cm square), located 60 cm above the floor. The screen was inclined to 45° with the rat facing downward. The time of turning face upward was recorded.
- Wire suspension. The rat was placed to hang by its front paws on a horizontal wire, approx. 60 cm above a foam cushion. The time until the rat fell from the wire was recorded.
- Bridges. Three flat bridges made of wooden plank (2, 4, 6 cm width) and one round (dowel, 2 cm in diameter), each 60 cm long suspended between two platforms, 60 cm above the cushion, were used. The rat was placed in the middle of the bridge. If the animal reached the platform within 60 s, the latency to reach the platform was the time in seconds taken to reach the platform, and the latency to fall was 60 s. If the rat fell, the latency to fall was the time in seconds until it fell, and the latency to reach the platform was 60 s.

As an index of visual acuity, the visual placing reflex, i.e. the response of an animal to extend its forepaws when lowered gently by the tail towards a flat surface, was used [23].

The behavioral tests, i.e. open field with an illuminated center (OF), elevated plus maze (EPM) and social interactions (SI) were performed once for each animal according to the following schedule: OF—3 days break—EPM—3 days break—SI. Before each trial in each test, the arena was cleaned with water containing a detergent and dried with a towel.

Video-based EthoVision System (Noldus, Wageningen, The Netherlands) recording spatiotemporal measures of a rat's movements was used to collect and analyze data of open field and plus maze tests.

### 2.3. Open field with illuminated center

The test arena was a black painted square ($95 \times 95$ cm) enclosed by walls (30 cm height), illuminated in the center by a 50 W halogen bulb suspended 30 cm above [39]. The animal was placed half way between the light

and border, and the track of its movements was recorded for 9 min. For further analysis, additional software (Track Explorer, Boguszewski, 1999) was used to define, on the basis of place preference plot for all rats, ''safe'' (peripheral) and ''unsafe'' (center) zones of the open field. Then, using EthoVision the following parameters were calculated: (1) in the whole arena: time of motor activity, distance moved and mean velocity; (2) in the central zone: total time spent in zone, number of entries into the zone, time of motor activity and distance moved; and (3) in the border zone: time of motor activity and distance moved. In addition, the ratio (percentage) of motor activity time/total time was calculated (named motor activity time/total).

### 2.4. Elevated plus maze

The black, wooden apparatus was based on that described by Pellow et al. [32] and consisted of two opposite open and closed arms (40 cm long and 10 cm wide, closed arms were enclosed by walls 40 cm high forming a cross with a $10 \times 10$ cm square center area). The apparatus was mounted 50 cm above the floor illuminated by a dim red light (40 W bulb). Each rat was placed into the center of the plus maze facing a closed arm. Spatiotemporal measures were recorded for 5 min. After the experiment, the borders between the central arena and both arms were defined (with the use of Track Explorer) for automatic EthoVision data analysis. The following parameters were calculated: (1) in the whole apparatus: time of motor activity, distance moved, total number of entries (an entry was counted when both forepaws were placed into the arm—this parameter was additionally verified by a human observer from the video tape); (2) in the central platform: total time spent; (3) in the closed arms: number of entries, time of motor activity inside the arm, total time spent and distance moved; and (4) in the open arms: number of entries, time of motor activity inside the arm, total time spent and distance moved. In addition, the ratio (percentage) of open arm entries relative to the total and ratio of motor activity time/total time were calculated.

### 2.5. Social interactions test

Each experimental rat was placed in the test arena ($50 \times 95$ cm, 30 cm high) for 10 min and then confronted for the next 10 min with a stranger rat of the same age and similar body weight. The encounters were videotape recorded for 10 min and then different behavioral events were encoded from the video recordings by two independent observers using a computer-based method (EthoLog, Boguszewski 2000). The data were then processed for each experimental rat. Each variable related to social behavior was a sum of corresponding behavioral events (measures):

(1) Number and (2) time of active pro-social episodes (including: approaches and chasing the partner, nose to nose contact, investigation, allogrooming, crawling over/under).
(3) Number and (4) time of passive pro-social episodes (including: head orienting, attention, cling close—the rats sitting or lying with their bodies in contact, but without interacting with each other).
(5) Number of withdrawal episodes (including: retreat, flight, evade).

Ambulation of non-social character, i.e. the number of crossings, was registered and analyzed as a sixth variable.

### 2.6. Statistics

In order to assess the performance of young (YA) and old (OA) rats in the sensorimotor tasks, the data were standardized for separate days and tasks and then summarized for each animal. For both groups, the distribution of individual scores was tested using Shapiro–Wilk's W test.

The results of each behavioral test were analyzed separately for YA and OA by factor analysis, using a principal components solution (PCA) with a varimax orthogonal rotation of the factor matrix. PCA ensures that the extracted factors are independent of one another and therefore reflect separate processes [34]. The number of factors extracted for each analysis was obtained on the basis of two criteria-Kaiser criterion, i.e. only factors with eigenvalues greater than 1 were left and the 75% variance rule, i.e. sum of eigenvalues exceeds 0.75. According to the Statistica for Windows Manual [36], the choice of the number of factors is arbitrary and in practice an important aspect is the extent to which a solution is interpretable. For data of the social interactions test, factors with eigenvalues greater than 0.9 were chosen. The factor loading indicates the correlation of each behavioral variable to each factor. Only factor loadings higher than 0.5 (or lower than $-0.5$) were reported.

In the next step, the variables found by PCA as the measures best reflecting the extracted factors were compared (for open field, EPM and social interactions, separately) by the Mann–Whitney rank test to assess the differences between the groups of young and old animals.

## 3. Results

### 3.1. Sensorimotor tasks

Sensorimotor tests were provided only to test the homogeneity of groups, i.e. eliminate animals with large

physical impairments. Shapiro–Wilk's W test for normal distribution showed that we cannot reject the hypothesis that a respective distribution is normal. For both groups, W statistics are insignificant (young rats $P < 0.8568$; old rats $P < 0.5531$).

### 3.2. Open field with illuminated center

Factor analysis of open field measures, made at first for 9 min of test duration, allowed the extraction of two factors for old rats, but only one for young animals. In OA, two independent factors with eigenvalues $>1$ representing 87% of the total variance emerged. Factor 1, on which the parameters measured in the whole arena and peripheral zone loaded highly, appeared to reflect motor activity. Factor 2, with high and exclusive loadings of the parameters measured in the central zone, appeared to reflect anxiety. In YA, all measures loaded highly on one factor representing 85% of the total variance (Table 1).

Factor analysis made for the first 3 min revealed, in both groups, two independent factors with eigenvalue higher than 1, representing 93.3% (YA) and 86.7% (OA) of the total variance (Table 2; Fig. 1). In both groups, factor 1 appeared to reflect motor activity with parameters measured in the whole arena and peripheral zone highly loading on this factor. Factor 2 appeared to reflect anxiety with high loadings of parameters measured in the central zone of arena.

Factor analysis confirmed that the parameter 'distance moved' measured in the peripheral zone was the variable best reflecting motor activity of the animal because it highly correlated with factor 1 and poorly correlated with factor 2 (Table 2). As shown in Fig. 2, this variable declined significantly ($P < 0.05$) with age. The parameter "time spent in central zone" was considered as the variable best reflecting the anxiety level (high correlation with factor 2, poor correlation with factor 1). As can be seen in Fig. 3, the time spent in the central zone decreased significantly with age ($P < 0.005$), indicating an increase of anxiety in OA compared with YA.

### 3.3. Elevated plus maze

In both groups, three independent factors with eigenvalues $>1$, representing 95.8% (YA) and 89.6% (OA) of the total variance, emerged from a factor analysis of the EPM measures (Table 3; Fig. 4). In young animals, factor 1, on which close arms activity loaded highly, was considered to reflect motor activity. The parameters measured in open arms all contributed to factor 2. Thus factor 2 appeared to reflect the anxiety level. As seen in Table 3, the order of the factors in old rats (the proportion of original variance represented by the factors) representing motor activity and anxiety was reversed, i.e. measures of anxiety loaded on factor 1 and measures of activity loaded on factor 2.

Factor 3, on which the time spent in the central platform of EPM loaded highly in both groups, was considered to reflect decision making processes (Table 3).

The number of entries to the closed arms declined significantly ($P < 0.001$) in OA, showing an age related decrease of motor activity (Fig. 5). Also, the ratio of open arm entries in relation to the total number of entries decreased significantly ($P < 0.001$) in OA, indicating a higher anxiety level in OA compared to YA (Fig. 6). Factor analysis confirmed that these measures are the best indicators of respective behaviors in young as well as old animals.

### 3.4. Social interactions test

In both groups, three factors with eigenvalues above 0.9, representing 84.2% (YA) and 85.8% (OA) of total

Table 1
Orthogonal factor loadings for open field measures in young and old rats (9 min test)

| | Young | Old | |
|---|---|---|---|
| | Factor 1 (85%) Motor activity | Factor 1 (60%) Motor activity | Factor 2 (27%) Anxiety |
| Distance moved in whole arena | 0.97 | 0.98 | |
| Distance moved in border zone | 0.89 | 0.99 | |
| Distance moved in central zone | 0.90 | | 0.92 |
| Number of entries into the central zone | 0.91 | | 0.71 |
| Total time spent in central zone | 0.80 | | 0.78 |
| Mean velocity in whole arena | 0.97 | 0.98 | |
| Motor activity time/total in whole arena | 0.98 | 0.97 | |
| Motor activity time/total in border zone | 0.93 | 0.99 | |
| Motor activity time/total in central zone | 0.94 | | 0.84 |

The percentage of the total variance accounted for by each factor is given in parentheses. Young rats accounting for 85% and old rats accounting for 87% of the total variance.

Table 2
Orthogonal factor loadings for open field measures in young and old rats (3 min test)

| | Young | | Old | |
|---|---|---|---|---|
| | Factor 1 (73%) Motor activity | Factor 2 (21%) Anxiety | Factor 1 (55%) Motor activity | Factor 2 (31%) Anxiety |
| Distance moved in whole arena | 0.94 | | 0.99 | |
| Distance moved in border zone | 0.98 | | 0.97 | |
| Distance moved in central zone | | 0.87 | | 0.95 |
| Number of entries into the central zone | 0.52 | 0.76 | | 0.72 |
| Total time spent in central zone | | 0.89 | | 0.73 |
| Mean velocity in whole arena | 0.93 | | 0.99 | |
| Motor activity time/total in whole arena | 0.92 | | 0.98 | |
| Motor activity time/total in border zone | 0.97 | | 0.98 | |
| Motor activity time/total in central zone | | 0.91 | | 0.88 |

The percentage of the total variance accounted for by each factor is given in parentheses. Young rats accounting for 94% and old rats accounting for 86% of the total variance.

variance, emerged from a factor analysis of the social test data (Table 4). There were, however, important differences in these factors between the two age groups. In young rats, factor 1 received the most significant contribution from the variables related to active social behavior, including pro-social as well as withdrawal attitudes. Factor 2 correlated with the number of episodes of clinging close, head orienting and attention directed to the partner, i.e. passive social behavior. The duration of these episodes loaded negatively on factor 2. Factor 3, on which the number of crossings loaded highly and exclusively appeared to reflect motor activity.

In old rats the, number and duration of active pro-social episodes and the number of passive social episodes contributed to factor 1. Factor 2, with the highest loading of the number of crossings, was considered as an index of motor activity. The duration of passive social episodes loaded negatively to this factor. Factor 3 received the most significant contribution from the variables associated with withdrawal from social contacts.

Mann–Whitney test showed no significant differences between old and young animals in the number and duration of active pro-social behavioral events. Also, there were no significant differences in the number of the episodes of withdrawal from social contacts (unsociable behavior) between groups.

A significant increase ($P < 0.001$) in the number and duration of passive social behavior was observed in OA compared to YA. Motor activity unassociated with the presence of partner decreased significantly ($P < 0.001$) in OA compared to YA. Typically agonistic events (of a

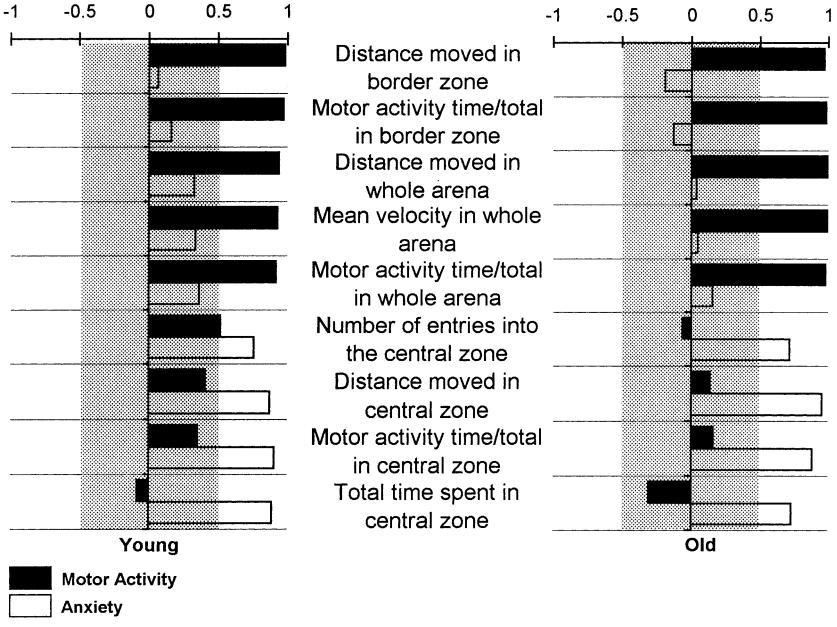


Fig. 1. Factor loadings for the measures in 3 min open field. Gray transparent rectangle covers factor loadings $< 0.5$.

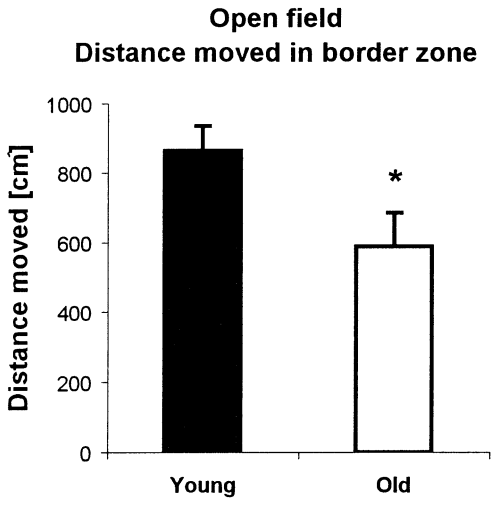


Fig. 2. Distance moved in peripheral zone during first 3 min of open field test (mean ± SEM). Significant difference (*) $P < 0.05$ compared to young rats group.

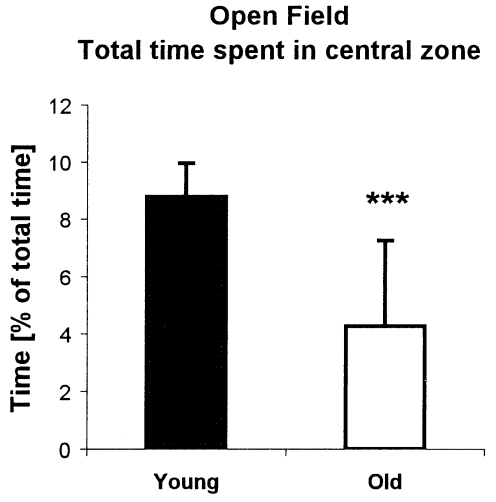


Fig. 3. Time spent in central zone during first 3 min of open field test (mean ± SEM) expressed as a percent of total test time. Significant difference (***) $P < 0.001$ compared to young rats group.

mild intensity) were only observed in a few cases, exclusively in the group of young animals.

## 4. Discussion

It has been demonstrated in many studies that motor activity in rodents declines with age [12,18,23,35]. There were also reports about an increased level of anxiety in old rats and mice [10,23]. However, it was unknown whether this effect results from the decrease of motor behavior, which is important since all commonly used tests include motor as well as an emotional component.

Our experiment proved that these two factors, i.e. anxiety and motor activity, predominantly influence the spontaneous behavior in open field and EPM and they are independent from each other in both age groups. Therefore, the increased level of anxiety found in old rats does not result from the decreased level of their motor activity.

Our data also showed qualitative changes in the social behavior of old rats when compared to young individuals.

Factor analysis revealed independent factors of anxiety and motor activity in the open field for both groups—YA and OA. It should be noted, however, that in young animals, two independent factors emerged only if the analysis was made for the first 3 min of the session. According to Lalonde and Badescu [21], motor activity at the beginning of the session may serve as a measure of exploration and information gathering since the animal moves around to investigate the new arena. As time passes, the animal habituates to the environment. Fraley and Springer [9] found that aging slows down the process of habituation to motor activity in the open field. Thus, the lack of differentiation between motor and emotional components in young animals might be due to the long duration (9 min) of the open field session.

The order of the factors reflecting motor activity (factor 1) and anxiety (factor 2) revealed in 3 min open field test was the same in young and old rats. However, in the latter group, the anxiety factor accounted for more of the total variance then motor activity. This suggests that the open field behavior of old animals is more related to anxiety than to motor activity, compared with young rats.

Factor analysis revealed not only two independent factors characterizing the behavior in the open field but it allowed the establishment of further comparison of the variables that best reflect them. As seen in Table 2 in the case of motor activity, the variable of highest correlation to this factor was the distance moved in the peripheral zone while in the case of the anxiety factor-time spent in the central zone.

The comparison of these variables between age groups showed a significantly higher level of anxiety and significantly lower motor activity in old rats compared with young animals.

Factor analysis of the behavior measured in the EPM revealed three independent factors for young and old animals. There were, however, important differences in the factor loadings on these measures and in the order of factors 1 and 2.

In young animals, factor 1 seemed to reflect motor activity since the measures traditionally used as indices of motor activity in EPM loaded highly on this factor. In agreement with previous results [5,34], our data indicate that the number of closed arms entries is the most reliable index of motor activity, since it loaded highly and exclusively on factor 1. The other measures with high and exclusive loadings were: ratio of motor

Table 3
Orthogonal factor loadings for EPM measures in young and old rats

| | Young | | | Old | | |
|---|---|---|---|---|---|---|
| | Factor 1 (63%) Motor activity | Factor 2 (25%) Anxiety | Factor 3 (8%) Decision making | Factor 1 (52%) Anxiety | Factor 2 (30%) Motor activity | Factor 3 (7%) Decision making |
| Total time spent in central platform | | | 0.94 | | | 0.88 |
| Distance moved in closed arms | 0.95 | | | | 0.94 | |
| Number of entries into closed arms | 0.88 | | | | 0.75 | |
| Total time spent in closed arms | | | −0.89 | −0.83 | | |
| Distance moved in open arms | | 0.96 | | 0.98 | | |
| Number of entries into open arms | | 0.87 | | 0.97 | | |
| Total time spent in open arms | | 0.94 | | 0.90 | | |
| Distance moved in whole apparatus | 0.75 | 0.53 | | | 0.95 | |
| Total numbers of entries | 0.61 | 0.63 | | | 0.67 | |
| Ratio open/total entries | | 0.89 | | 0.96 | | |
| Motor activity time/total in closed arms | 0.96 | | | | 0.81 | |
| Motor activity time/total in open arms | | 0.95 | | 0.99 | | |
| Motor activity time/total in whole apparatus | 0.68 | | 0.57 | | 0.87 | |

The percentage of the total variance accounted for by each factor is given in parentheses. Young rats accounting for 96% and old rats accounting for 89% of the total variance.

activity time in closed arms/total time spent in closed arms, and distance moved in closed arms, which were recorded in our study additionally to conventional measures. These measures may therefore serve as good indices of motor activity in EPM. The total number of arm entries loaded considerably on factor 1 as well as on factor 2, which confirms the notion of several other authors [5,8,28,34] that this measure provides a con-

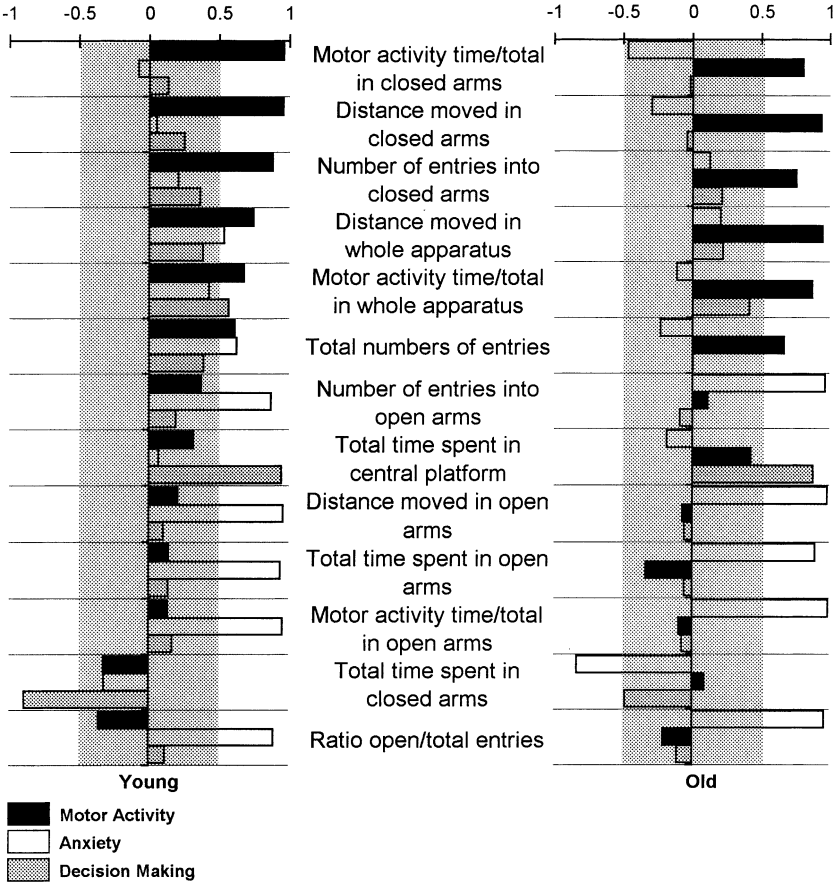


Fig. 4. Factor loadings for the measures in EPM. Gray transparent rectangle covers factor loadings $< 0.5$.

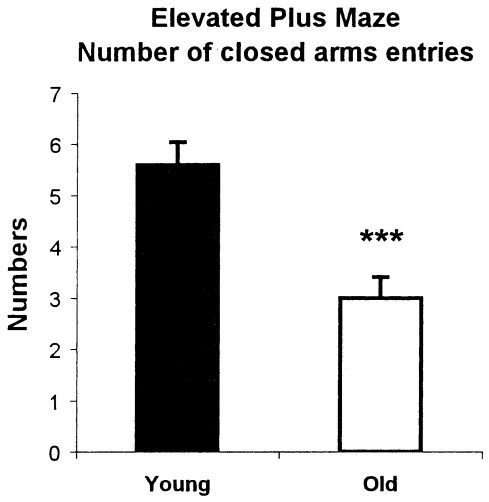


Fig. 5. Number of entries to the closed arms in EPM (mean ± SEM). Significant difference (***) $P < 0.001$ compared to young rats group.

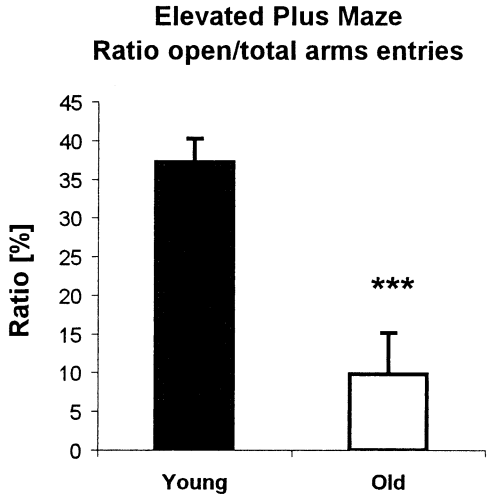


Fig. 6. Ratio of open arm entries in relation to the total number of entries in EPM (mean ± SEM). Significant difference (***) $P < 0.001$ compared to young rats group.

taminated index. Factor 2 in young rats was considered an index of anxiety, since open arm activities and open/total entry ratio loaded highly and exclusively on this factor. In addition to the total/open ratio reported by many authors as the best index of anxiety, our analysis revealed other variables highly and exclusively loading on this factor, i.e. distance moved and time spent in open arms, and ratio of motor activity time/total time spent in open arms. Again, they may therefore serve as useful measures of anxiety in EPM.

Besides the anxiety and motor activity factors, the analysis also revealed a third independent factor, most probably related to decision making, on which the time spent at the central platform loaded highly. This finding is in agreement with the results of other authors who included central platform time into the analysis of EPM behavior [5,8,34]. As in the Rodgers and Johnson [34] study, the time spent in closed arms loaded highly, but negatively on factor 3, although only in young rats.

In old animals, the order of factors separately reflecting motor activity and anxiety was reversed so that measures of activity loaded on factor 2, while measures of anxiety loaded on factor 1. Also, a much greater percent of the total variance was accounted by the factor reflecting anxiety in OA than in YA. These data suggest that the behavior of old individuals in the EPM was mostly driven by the anxiety component. Measures of open arm activity loaded highly on factor 1. The time spent in closed arms loaded highly but negatively on this factor. This indicates that the time in the closed arms in old rats is correlated with their anxiety level, while the same measure in young animals is related to the process of decision making. The time spent on the central platform in OA loaded highly and exclusively on factor 3, seemingly reflecting decision making.

The comparison of the most reliable indices of anxiety level (open/total ratio) and motor activity (number of closed arm entries) significantly demonstrated a lower motor activity and significantly higher level of anxiety in old rats compared with young rats.

Three independent factors emerged from the analysis of social interaction measures. The pattern of factor loadings was different for young and old animals, except for the motor activity factor, on which the number of crossings loaded highly at each age. In young rats, factor 1 was related to active social behavior in its full dimension. Measures of pro-social as well as withdrawal attitudes loaded highly to this factor. Factor 2 seemed to represent passive social behavior with high and exclusive loading of the number of passive events and time spent on passive interactions (with negative sign). Factor 3 appeared to reflect motor activity.

In old rats, factor 1 showed a high loading for the number of active social events and the time spent on active interactions. Also, the number of passive events loaded highly on this factor. Factor 2 appeared to represent motor activity. The time of passive events also loaded highly but negatively to this factor. This might indicate that the passive form of interaction in old rats is rather related to motor activity than to social behavior per se. Factor 3, which is particularly interesting, received the most significant contribution from the variable representing withdrawal from social contacts, i.e. unsociable behavior.

The comparison of the number and time spent on social interactions did not show age-related differences. Factor analysis indicated, however, that the "structure" of social behavior is distinct in OA compared with YA. It might be supposed that factor 1 in both YA and OA referred to social motivation, but while in YA it represented the tendency to active pro-social contacts on one side and the tendency to withdrawal from

Table 4
Orthogonal factor loadings for social interaction measures in young and old rats

| | Young | | | Old | | |
|---|---|---|---|---|---|---|
| | Factor 1 (44%) Active social behavior | Factor 2 (22%) Passive social behavior | Factor 3 (18%) Motor activity | Factor 1 (41%) Social behavior | Factor 2 (30%) Motor activity | Factor 3 (15%) Unsociable behavior |
| Ambulation of non social character | | | 0.99 | | 0.94 | |
| Time of passive pro-social episodes | | −0.86 | | | −0.73 | 0.55 |
| Time of active pro-social episodes | 0.83 | | | 0.96 | | |
| Number of active pro-social episodes | 0.87 | | | 0.82 | | |
| Number of passive pro-social episodes | −0.52 | 0.69 | | 0.85 | | |
| Number of withdrawal episodes | −0.91 | | | | | 0.94 |

The percentage of the total variance accounted for by each factor is given in parentheses. Young rats accounting for 84% and old rats accounting for 86% of the total variance.

interactions on another, in OA this polarity did not exist—active pro-social time and events correlated with the number of passive episodes. Withdrawal tendency, the motivation to avoid interactions, was represented by an independent factor (factor 3) in old rats. This might be associated with their hyperemotionality proved in OF and EPM tests.

It was previously found that studies involving different models of anxiety fail to produce a unique anxiety factor [1,8,15,33]. File [8] and Belzung and Pape [1] reported that different measures of anxiety recorded in the EPM, Vogel test, social interactions test and others yield separate anxiety factors. In our attempt to analyze measures from both the EPM and social interactions test, the total/open arms ratio and the number of withdrawal episodes did not load on the same axis, thereby suggesting that they may relate to different types or aspects of anxiety. Further studies are needed to verify this supposition.

In our experiment, aggressive episodes (neither defensive/offensive postures nor attacks) were unobserved during social encounters, which is probably due to the fact that the animals in the present study were socially housed. According to Blanchard et al. [3], rats housed in stable social groups are much less likely to exhibit aggressive tendencies. The duration of such experiences might affect the pattern of social interactions. In our experiment, OA were socially housed for 2 years, whereas the period of stable socialization was much shorter for YA. Thus, it should also be taken into consideration that different socialization periods might cause, or at least contribute to, the distinct structure of social behavior in old versus young animals as revealed by factor analysis.

The results of our study both confirmed and extended earlier observations indicating age-related changes in emotional behavior in rats [4,10,24,31]. The factor analysis allowed the definition of various motivational factors that underlie spontaneous behavior of young and old rats in commonly used tests and identified the relationships between specific test indices. The PCA approach helped the assessment of the validity of the tests. This is necessary when comparing two age groups because the differences between animals of different age might not only be quantitative but also qualitative—as pointed out by Giuliani et al. [14] in their multivariate analysis of behavioral aging.

Factor analysis proved especially useful in the analysis of more complex behavior, i.e. social interactions. As we have shown, the simple comparison of means did not find significant differences in social behavior between old and young animals, whereas factor analysis demonstrated that the pattern of social encounters is distinct in OA compared to YA. Further studies are needed to answer the question whether this difference is related to the changes in emotionality or to the long lasting socialization experience in old animals.

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