Adult Wistar Rats

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Rats are considered adults from 2 to 5 months. During this period, they are used for experimentation in physiology and pharmacology. Adult rats, depending on their age, can be in a different physiological state, which can influence the results of experiments carried out on them. Despite this, age-related changes in adult rats have not yet been examined. Our results showed that as male and female rats progressed from 2 to 5 months of age there was a decrease in the level of motor and exploratory activities, and an increase in the level of anxiety-like behaviour. Age-related changes were dependent upon initial individual characteristics of behaviour. For example, animals that demonstrated high motor activity at 2 months become significantly less active by 5 months, and animals that showed a low level of anxiety at 2 months become more anxious by 5 months. Low-activity and high-anxiety rats did not show any significant age-related changes from 2 to 5 months of age.

Keywords: age ; behaviour ; open field ; physical activity

1. Introduction

Approximately 80% of the experimental non genetically modified animals used for physiological and pharmacological studies are mice, rats, and guinea pigs [1][2]. Rats are the animals most frequently used in scientific research [3]. Moreover, rats are frequently used to study the mechanisms underlying the functioning of the nervous system, as well as in behavioural studies [4].

Currently, one of the most widely used lines of laboratory animals is the Wistar rat. A significant number of modern laboratory rat lines have been developed from this line, including Sprague Dawley and Long Evans rats. It is known that various behavioural characteristics of rats change with age. The average lifespan of a rat is about 3 years [5]. Rats develop rapidly and their adolescence ends by the end of the second month of ontogenesis. Thus, a rat at 2 months of age (60 days) is considered an adult [2][5][6][7]. By this time, all the vital systems of the rat's body have matured. The social maturity of rats occurs between the ages of 5 and 6 months [2][5]. After 6–7 months, rats begin to show signs of ageing. As each individual grows and develops, its behaviour also changes.

In rodents, age-related changes in behaviour are usually investigated by comparing the corresponding indicators of young and old animals. Compared to younger rats, a number of studies have reported that older rats have a decrease in behavioural characteristics associated with sensory, motor, and cognitive functions [8][9][10]. When studying the behaviour of rats in the elevated plus maze (EPM) test, it was found that older rats showed a higher level of anxiety [11]. Testing rats of different ages in the Morris water maze showed a decrease in cognitive function in elderly compared to young rats [12]. It was previously shown that adolescent rats had a higher level of anxiety in the EPM and open field (OF) tests compared to adult animals [13]. However, although most physiological and pharmacological studies are conducted in adult rats (2 to 5 months), there has not yet been a specific study of the behavioural changes that occur during this period of a rat's lifespan.

It has been shown that rats, although identical in lineage (breed), sex, age, and conditions of housing, can individually differ significantly in terms of behaviour and physiology [14][15]. Such individual differences can be quite stable and can be detected using behavioural screening tests [16][17][18][19]. It has been shown that initial individual differences can influence the formation of age-related changes in behaviour [20][21][22]. However, these data are relevant to old, but not younger (i.e., aged 2–5 months) adult rats.

2. Age-Related Changes in Adult Rats

It was found that in 5-month-old males who performed behavioural tests for the first time (first group) in comparison with 2-month-old males, there was a significant decrease in motor activity as measured by the EPM ($U = 116.50$; $Z = 3.29$; $p = 0.00995$) and OF ($U = 74$; $Z = 4.20$; $p = 0.0003$) tests. In 5-month-old males who were tested a second time (second

group), there was also a decrease in motor activity (U = 155; Z = 3.61; p = 0.0003) and a decrease in the number of rearings (U = 92.50; Z = 4.69; p = 0.000003) in the EPM, and a reduction in motor activity (U = 63.50; Z = 5.20; p = 0.001) and number of rearings (U = 203.50; Z = 2.77; p = 0.005) in OF (**Figure 1**A,B).

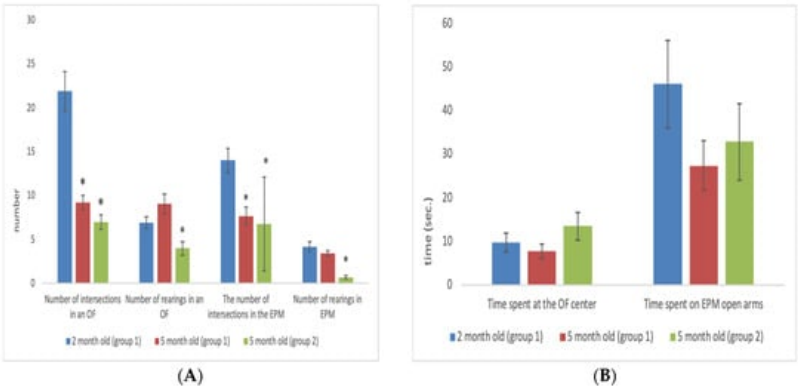

Figure 1. (**A**,**B**). Behavioural activity of males at the ages of 2 and 5 months. ***** p < 0.05 (2-month-old vs. 5-month-old). (**A**): Values in numbers. (**B**): Values in seconds.

In females, by the fifth month, a significant decrease in motor activity in the OF test (U = 46; Z = 3.65; p = 0.0002) and a decrease in the number of rearings in the EPM (U = 36.50; Z = 3.65; p = 0.00008) were observed (**Figure 2**A,B).

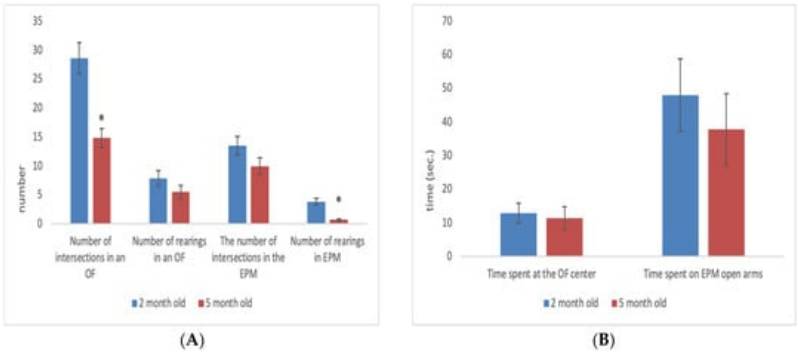

Figure 2. (**A**,**B**). Behavioural activity of females at the ages of 2 and 5 months. ***** p < 0.05 (2-month-old vs. 5-month-old). (**A**): Values in numbers. (**B**): Values in seconds.

Since there was little variation in OF and EPM results at the age of 5 months in males in the first group compared to males in the second group at the same age, further analysis of age-related changes in the same animals was carried out during the initial testing at the age of 2 months and retesting at the age of 5 months. In females, comparison of single and repeated testing was not carried out.

3. Age-Related Changes in Rats with High and Low Motor Activity in the OF and EPM Tests

For males, locomotor activity decreased with age, as determined by the OF (U = 0.01; Z = 2.93; p = 0.003) and EPM (U = 4; Z = 2.55; p = 0.01) tests. No significant differences were found in the level of anxiety. For males that were passive in the OF test, no significant age-related changes were observed except for a decrease in the number of rearings in the EPM (U = 5.0 Z = 2,427,731 p = 0.015194)

In active females, motor activity decreased with age in the OF test (U = 0.01; Z = 3.06; p = 0.002). However, these changes were less pronounced than in males. In females that were passive in the OF, only a decrease in the number of rearings in the EPM was noted (U = 7.50 Z = 2,108,293 p = 0.035006)

The active and passive males in the OF test at the age of 2 months differed only in the level of horizontal (U = 0.01; Z = −3.07; p = 0.02) and vertical activity (U = 0.01; Z = −3.06; p = 0.002) (Table 1). At the age of 5 months, these differences diminished. In active and passive females at the age of 5 months, the differences observed at 2 months in the level of motor activity in the OF (U = 0.01; Z = −3.06; p = 0.002) also receded (Table 2).

In males that were active in the EPM, a significant decrease with age in motor activity was also observed, both in the OF (U = 0.01; Z = 3.06; p = 0.002) and in the EPM (U = 0.01; Z = 3.06; p = 0.002). The number of rearings also decreased in the OF (U = 8; Z = 2.04; p = 0.04) and in the EPM (U = 1.0; Z = 2.9; p = 0.003) tests. There were no age-related differences in the level of anxiety.

In males that were passive in the EPM, no significant age-related changes in the levels of motor and exploratory activities were

In females active in the EPM, motor activity significantly decreased with age in the OF (U = 7.50; Z = 2.11; p = 0.03) and EPM (U = 1; Z = 2.94; p = 0.003) tests, and the number of rearings in the EPM decreased (U = 2; Z = 2.81; p = 0.005). No significant age-related changes were observed in passive females

Males, both active and passive in the EPM at the age of 2 months significantly differed in the number of intersections of the maze compartments (U = 0.01; Z = −3.07; p = 0.002). At 5 months, these differences were not apparent.

EPM motor activity in females at 2 months was significantly greater compared to passive females (U = 0.01; Z = −2.93; p = 0.003). They also spent longer time in the open arms (U = 1.50; Z = −2.71; p = 0.007) than rats that were passive in the EPM. At 5 months, these differences between active and passive females in the EPM were not apparent.

4. Age-Related Changes in Rats with High (HA) and Low Levels of Anxiety (LA) in the OF and EPM Tests

Despite the fact that we did not reveal age-related differences in the level of anxiety in the general population of Wistar rats, LA in the OF test males, at the age of 2 months, demonstrated a low level of anxiety (spent a longer time in the centre of the OF), and had a significantly increased level of anxiety by the fifth month (U = 11.50; Z = 2.10; p = 0.036). In addition, in LA males in the OF, by the fifth month, motor activity decreased both in the OF (U = 0.01; Z = 3.31; p = 0.0009) and in the EPM (U = 7; Z = 2.57; p = 0.01) tests, and the number of rearings in the OF (U = 8.50; Z = 2.41; p = 0.02) and in the EPM (U = 8; Z = 2.47; p = 0.01) also decreased (Table 5). Those males that demonstrated a high level of anxiety at age 2 months had no reliable age-related changes in levels of motor activity and anxiety. We observed only a significant decrease in the number of rearings in the EPM (U = 3.0; Z = 2.68; p = 0.007).

In both LA and HA in the OF test females, no significant age-related changes in the studied parameters were observed, except for a decrease in the number of rearings in the EPM in calm (U = 3.5; Z =2.62; p =0.009) and in anxious animals (U =7.5; Z = 2.11; p = 0.035)

The observed significant differences between LA and HA in the OF test in males at the age of 2 months in motor activity (U =1; Z = −3.07; p = 0.002), the number of rearings (U =5.5; Z = −2.55; p = 0.01), and the time spent in the centre of the arena (U = 0.01; Z = −3.18; p = 0.001) was not present by the fifth month. The observed significant differences between LA and HA 2-month-old females in terms of the time spent in the centre of the OF (U = 0.01; Z = −3.07; p = 0.002) was also not apparent at the age of 5 months.

Males who, at the age of 2 months, demonstrated a low level of anxiety in the EPM (prolonged time spent in open arms), by 5 months significantly reduced the time spent on open arms (U = 7.0; Z = 2.17; p = 0.03). Motor and exploratory activity of LA in EPM males, both in the EPM (U = 7.5; Z = 2.11; p =0.035) and in the OF (U = 1; Z = 2.94; p = 0.003) also significantly decreased with age. In males that demonstrated a high level of anxiety at the age of 2 months, the number of rearings in the EPM decreased with age (U = 2.5; Z = 2.75; p = 0.006). No other significant age-related changes in behaviour were observed In males that demonstrated a high level of anxiety in the EPM at the age of 2 months, practically no significant age-related changes in behaviour were observed.

Females showing a low level of anxiety in the EPM at the age of 2 months also significantly reduced the time spent on open arms by 5 months (U = 4.0; Z = 2.555; p = 0.01). In addition, with age, they had a decrease in the number of rearings in the EPM (U = 2.5; Z = 2.75; p = 0.006). In females showing a high level of anxiety in the EPM, by the fifth month the time spent in the open arms did not change. However, the number of rearings in the EPM significantly decreased (U = 3.0; Z = 2.68; p = 0.007)

Males with high and low anxiety in the EPM differed at the age of 2 months in the duration of stay in the open arms of the maze (U = 0.01; Z = −3.07; p = 0.002). By the fifth month, significant differences between groups were not observed.

Females with high and low anxiety in the EPM at 2 months significantly differed in the time spent on the open arms of the maze (U = 0.0 1; Z = −3.07; p = 0.002), as well as in the level of motor activity in the EPM (U = 3; Z = −2.68; p = 0.007). These differences were not found at the fifth month.

5. Conclusions

4. In male and female Wistar rats there is a decrease in the level of motor and exploratory activities from 2 to 5 months of age.
5. Age-related changes in adult Wistar rats depend on their initial individual characteristics of behaviour.
6. Animals that demonstrate high motor activity at 2 months become significantly less active by 5 months.
7. Animals that show a low level of anxiety at the age of 2 months become more anxious by 5 months.
8. Low-activity and high-anxiety rats do not exhibit age-related changes in OF and EPM tests from 2 to 5 months of age, except for a decrease in the number of rearings in the EPM.

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