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Remembering how to run: A descriptive wheel run analysis in CF1 male and female mice

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ABSTRACT

Physical exercise is known to have beneficial effects on general health and wellbeing in humans and it is also related to neuronal plasticity, increasing neurogenesis and consequently leading to improvements in processes such as learning and memory. In this sense, wheel running performance in mice appears as an extensively used behavioral approach for neurobiological studies. Here, we explored the running patterns in CF1 male and female mice allowing voluntary wheel running for 20 min along three consecutive days. We analyzed differences in the accumulated distance traveled, instant velocity, and latency to run and breaks taken in both males and females, comparing performance between days. Results revealed that after a first experience with the wheel, animals that had learnt how to run on day 1 quickly look forward to stepping into the wheel in subsequent training days, reflected by a significant increase in daily running distance and velocity. Further, no differences were found in the running performance between males and females. In summary, in a first experience with the wheel, animals get familiarized with the wheel and grow accustomed to it.

1. Introduction

Physical activity is widely known for its beneficial effects on general health and wellbeing in humans when practiced regularly (Löllgen 2013; Piepoli et al., 2016; Mueller et al., 2017). Furthermore, it has been proved to be an effective non- pharmacological therapy for many diseases (Löllgen, 2013). Furthermore, an increasing body of evidence indicates that physical activity and exercise training can induce remarkable functional and neuroanatomical plasticity including neurogenesis, angiogenesis, synaptic plasticity and dendritic morphological remodeling. Exercise-induced neuroplastic changes are thought to play critical roles in mediating important beneficial effects associated with physical activity, including improved memory, cognitive function and neuroprotection, among others (Dishman et al., 2006; Mueller, 2007; Cotman et al., 2007; van Praag, 2008; Baruch et al., 2004; Burghardt et al., 2004; Van Hoomissen et al., 2004; O'Callaghan et al., 2007; Clark et al., 2008; Kohman et al., 2011; Gomes da Silva et al., 2012). These changes are known to be mediated by an increase of brain derived neurotrophic factor (BDNF), fibroblast growth, and insulin- like growth factors, as well as increases in acetylcholine, opiate, and monoamine neurotransmitters (Neeper et al., 1996; van Praag et al., 1999b, 1999a; Greenwood et al., 2003; Lou et al., 2008; Liu et al., 2008; Greenwood and Fleshner, 2011; Lin and Kuo, 2013). Moreover, neuroplasticity induced by physical exercise is not only associated with memory processes but also linked to rewarding properties as it has been found that it produces plasticity in the mesolimbic reward pathway (Greenwood et al., 2011). Therefore, animal studies examining specific aspects or mechanisms underlying human physical exercise are one of the top interests in biomedical research. Hereby, voluntary wheel running in mice is not only an interesting model used to assess the beneficial effects of physical exercise on human health (de Visser et al., 2007) but also appears as an important tool for the improvement of processes such as learning and memory (van Praag et al., 1999a; Ang et al., 2006; Liu et al., 2009; Berchtold et al., 2010; Lin et al., 2012; Cardoso Cassilhas et al., 2016).

A wide range of behavioral effects of voluntary wheel running had

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been described (Lee et al., 2012; Balbus et al., 2013), including the neurobiology underlying physical activity (Garland et al., 2011) and translational studies to model the effects of exercise on health, brain, and behavior (Patterson et al., 2008; Haskell-Luevano et al., 2009). Voluntary wheel running might be satisfying some behavior such as playing, escaping, or metabolic drives, more related to a rewarding behavior and not to a classic stereotypic response that can result from environmental restriction (Banjanin and Mrosovsky, 2000; Garland et al., 2011; Meijer and Robbers, 2014). Moreover, not only laboratory mice run spontaneously when they have access to running wheels (Sherwin, 1998; Visser et al., 2005), but also free-ranging wild mice when running wheels are placed in nature (Meijer and Robbers, 2014).

Under this framework, a better understanding of the biological moderators of exercise is needed to deepen its role as a behavioral tool to maintain and promote brain health (Barha et al., 2017). Hence, it takes relevance to behaviorally characterize and understand the patterns and variability of wheel running in mice, as well as the factors that can affect voluntary running activity. Voluntary wheel running activity is strongly influenced by strain, sex, the design of the running wheel, environment conditions, among others, which consequently may impact the voluntary running activity (De Bono et al., 2006; Coletti et al., 2013; Triviño-Paredes et al., 2016). In this sense, sex arises as an important variable when assessing the effects of physical exercise in promoting healthy cognitive aging. Although there has been a recent growing body of research that compares exercise between females and males; still most of the studies evaluate the effects of physical exercise only in males (Allen et al., 2001; de Visser et al., 2007; Garrett et al., 2012; Goh and Ladiges, 2015). Knowledge of sex-related running profiles of laboratory mice is certainly useful for biomedical research looking at the effect of physical exercise on specific aspects that are assessed in each particular experiment. Further, since exercise is known to promote brain neuroplasticity, therefore it would be of high interest to evaluate its effects on memory studies. Thus, to better understand the effects of exercise in cognitive abilities, it first appears relevant to shed light on the running profile in mice to further explore the modulatory effect of exercise in memory paradigms.

Here we aim to characterize wheel running in females and males CF1 mice, commonly used in our lab in different behavioral assays including learning and memory tasks. To explore the pattern of running behavior, mice were placed on the wheel cage allowing voluntary running for 20 min along three consecutive days. We observed a significant increase in daily running distance along days and velocity, reaching a plateau in the final minute's animals spent running in both males and females. Overall, these results suggest that in a first experience with the wheel, animals familiarize and grow accustomed to it. This is evidenced by the fact that they run faster and cover longer distances in the following days. Moreover, animals that learnt how to run on day 1 quickly look forward to stepping into the wheel in subsequent training days, as there was a significant decrease in the latency to step into the wheel.

2. Materials and methods

2.1. Subjects

CF-1 adult males and females (N = 60, Age 60–70 days, weight 25–30 g) mice were used for the experiments. All mice used were from our own breeding stock. Animals were caged in groups of 8–10 in ventilated home cages (cage size: 50 × 30 × 15 cm) with ad libitum access to food and water. The housing room was kept on a 12-h light–dark cycle with lights on from 07:00 to 19:00 h and temperature regulated (23–25 °C) environment. The experiments were conducted between 08.00 and 14.00 h and were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No 80-23/96) and local guidelines establish by CICUAL-FFyB (CUDAP # 0044975-2016). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Wheel run apparatus and behavioral procedure

The wheels used for voluntary running, consisted in a plastic mouse running wheel (13 cm circumference), adapted with a hall effect sensor that detects the passage of a magnet attached to the wheel, plugged to an Arduino, and then connected to a computer setup.

Five wheel-cages were built allowing registering the behavioral activity of five animals simultaneously. Wheels were installed into clean empty cages, with wires attached to the bottom of the cage and then filled with bedding material. The feeding rack (no food available during the running) was placed above the wheel cage in a position that did not impede wheel movement but prevented animals from escaping. A program interface developed in MATLAB (version R2016a; The Mathworks, Inc.) allowed to record the total number of wheel revolutions in 10 s bins.

One experiment with two conditions was performed. 30 females and 30 males' animals were allowed to freely run for 20 min in three consecutive days. When the run time limit was reached, each animal was returned to his home cage until the next day. Wheel assignment was pseudo-randomized for all mice. Cages and wheels were cleaned before and after each experimental subject during the three-day experiment. Distance, instant velocity (with the graph of position versus time, instantaneous velocity was estimated as the slope of the tangent line at each interval point) mean velocity, latency to run, number of breaks and total breaks time were determined from the collected data.

2.3. Statistical analysis

2.3.1. Sample size and selection criteria

All the experiments carried out in this work started with 30 animals per experimental group. After analyzing the running behavior along the 3-day wheel-exposure protocol animals that did not reach at least 100 revolutions (40 m) or that took more than 400 s to start running on the first day of wheel exposure were considered outliers. These animals represented 2 standard deviations away from the group mean being excluded from the analysis. Consequently, the final number of animals per group was 26 females and 24 males.

2.3.2. Data analysis

All data sets were tested for a Gaussian distribution with the Shapiro-Wilks normality test. Distance and velocity followed a normal distribution. Thus, performance along the three consecutive running days were analyzed with a repeated measure Two-way ANOVA, followed by Tukey's multiple comparisons post hoc test and data was expressed as mean with the standard deviation. In the case of latency to start running, number of breaks and total time breaks, data did not adjust to a normal distribution being the performance along the three consecutive running days analyzed with the non-parametric Friedman test (for comparison of repeated measures performed to the same group). In this case data was expressed as median and interquartile range. For comparisons among groups of males and females a non-parametric Krustal-Wallis test was performed and the differences between groups were estimated by the non-parametric post hoc Dunńs test. Value of the statistic parameter (H for Kruskal-Wallis, Q for Friedman, and z for Dunn) is provided for each comparison, when appropriate. In all cases P values less than 0.05 were considered significant.

A correlation analysis using the Pearson coefficient was performed between the distance traveled and the number of breaks/total breaks time.

3. Results

3.1. Distance traveled and instant velocity: growing the habit

To explore running behavior, mice were placed on the wheel cage allowing voluntary running for 20 min along three consecutive days. We

first aimed to explore differences in the accumulated distance traveled in males and females, comparing performance in the same day along time and between days (Fig. 1 A-B). In females, repeated measure two way-ANOVA revealed significant differences in distance between day 1-day 2, day 1 - day 3 but not between day 2 - day 3 (Fig. 1A) [$ANOVA_{time}$: $F_{119,46} = 246.0$, $p < 0.0001$; $ANOVA_{Day}$: $F_{2,78} = 17.81$, $p < 0.0001$; multiple comparisons: Day1 vs Day 2: $p = 0.002$; Day 1 vs Day 3 $p < 0.0001$; Day 2 vs Day 3: $p = 0.2957$, d= 0.25]. When evaluating the interaction between day and time, significant differences between days for each time interval were found between day 1 and day 2 ($p = 0.0342$) and between day 1 and day 3 ($p = 0.0092$) from the 180 min time interval. No differences were found between day 2 and day 3 along all time intervals [Interaction Day x Time $F_{238,92} = 12.58$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p = 0.0342$; Day 1 vs Day 3: $p = 0.0092$; Day 2 vs Day 3: $p = 0.8941$].

On the other hand, males showed a similar pattern of increase in the traveled distance (Fig. 1. B) [$ANOVA_{time}$: $F_{119,32} = 534,9$, $p < 0.0001$; $ANOVA_{day}$: $F_{2,54} = 11.49$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p = 0.0185$; Day 1 vs Day 3 $p < 0.0001$; Day 2 vs Day 3: $p = 0.1329$, d= 0.34]. In males, the interaction factor between day and

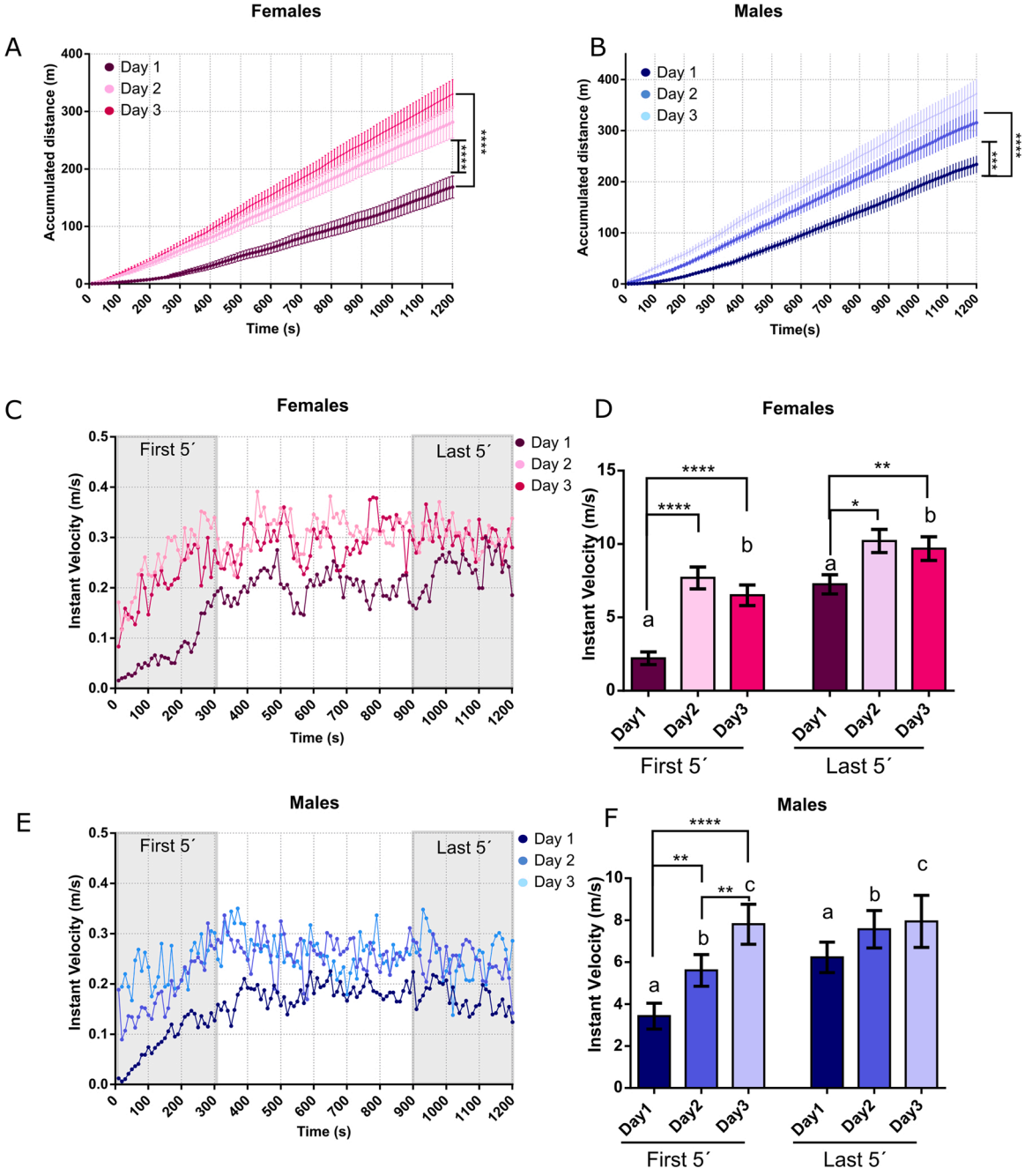

Fig. 1. Wheel running performance between days comparing males and females' performance. A. Accumulated distance female mice: Day1 vs Day 2: $p = 0.002$; Day 1 vs Day 3: $p < 0.0001$; Day 2 vs Day 3: $p = 0.2957$. B. Accumulated distance male mice: Day 1 vs Day 2: $p = 0.0185$; Day 1 vs Day 3: $p < 0.0001$; Day 2 vs Day 3: $p = 0.1329$. C. Instant velocity of female mice. First 5 min: Day 1 vs Day 2: $p < 0.0001$; Day 1 vs Day 3 $p < 0.0001$; Day 2 vs Day 3: $p = 0.3075$ Last 5 min: Day1 vs Day 2: $p = 0.9999$; Day 1 vs Day 3 $p = 0.9976$; Day 2 vs Day 3: $p = 0.9986$. D. Accumulated instant velocity for the first and last 5 min for female mice: Day 1 first vs last: $p < 0.0001$; Day 2 first vs last: $p = 0.1349$; Day 3 first vs last: $p = 0.0024$ E. Instant velocity of male mice. Fist 5 min: Day1 vs Day 2: $p = 0.0165$; Day 1 vs Day 3 $p < 0.0001$; Day 2 vs Day 3: $p = 0.0002$. Last 5 min: Day1 vs Day 2: $p = 0.6460$; Day 1 vs Day 3 $p = 0.133$; Day 2 vs Day 3: $p = 0.1492$. F. Accumulated instant velocity for the first and last 5 min for male mice. Day 1 first vs last: $p < 0.0001$; Day 2 first vs last $p < 0.0001$; Day 3 first vs last: $p = 0.0112$.

time revealed differences between day 1 and day 2 and between day 1 and day 3 from the 240 min time interval but no differences were found between day 2 and day 3 along all time intervals [Interaction Day x Time F238,64 = 5.22, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2 t: $p = 0.0432$); Day 1 vs Day 3: $p < 0.0001$; Day 2 vs Day 3: $p = 0.1030$].

Further, instant velocity reached in males and females across days was analyzed. A Two-Way repeated measure ANOVA was performed. In this case performance during accumulated time for the first and the last five minutes of wheel exposure was compared. Females showed an increase in instant velocity between days during the first five minutes [$ANOVA_{time}$ first 5 min: $F_{2.50} = 6.823$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p < 0.0001$, d= 1.83; Day 1 vs Day 3 $p < 0.0001$, d= 1.48; Day 2 vs Day 3: $p = 0.3075$, d= 0.24] but not differences were observed between days in the last five minutes [$ANOVA_{time}$ last 5 min: $F_{2.50} = 6.823$, $p < 0.0001$; multiple comparisons: Day1 vs Day 2: $p = 0.9999$, d= 0.02; Day 1 vs Day 3 $p = 0.9976$, d= 0.01; Day 2 vs Day 3: $p = 0.9986$, d= 0.009]. Moreover, differences between the first and the last five minutes were only observed for day 1 and day 3 [$ANOVA_{velocity}$: Interaction Day x Time $F_{2.50} = 23.00$, $p < 0.0001$, d= 0.80; multiple comparisons: Day $1_{first\ vs\ last}$: $p < 0.0001$; Day $2_{first\ vs\ last}$: $p = 0.1349$, d= 0.16; Day $3_{first\ vs\ last}$: $p = 0.0024$, d= 0.76] (Fig. 1. C-D).

When analyzing males performance, a significant increase in velocity during the first five minutes across days was observed [ANOVA: $F_{2,44} = 3.329$, $p < 0.0001$; multiple comparisons: Day1 vs Day 2: $p = 0.0165$, d= 0.38; Day 1 vs Day 3 $p < 0.0001$, d= 0.92; Day 2 vs Day 3: $p = 0.0002$, d= 0.53]. However, no differences were found in the last five minutes that animals spent running across days [$ANOVA_{velocity}$: $F_{2,44} = 3.329$, $p < 0.0001$; multiple comparisons: Day1 vs Day 2: $p = 0.6460$, d= 0.16; Day 1 vs Day 3 $p = 0.133$, d= 0.39; Day 2 vs Day 3: $p = 0.1492$, d= 0.25]. When evaluating the interaction between day and time, differences between the three days were found for velocity [Interaction Day x Time $F_{2.50} = 23.00$, $p < 0.001$; multiple comparisons: Day1 first vs last: $p < 0.0001$, d= 0.86; Day 2 first vs last $p < 0.0001$, d= 1.30; Day 3 first vs last: $p = 0.0112$, d= 0.56] (Fig. 1. E-F). Thus, curve analysis indicated a significant increase in daily running distance along days and instant velocity reaching a plateau in the final minute that animals spent running.

3.2. Total distance, mean velocity and latency to start running: parameters that matter

A two-way ANOVA was performed to analyze effect of sex and time (running day) on the total accumulated distance and velocity throughout each day. Results showed no significant interaction between sex and time day [$ANOVA_{distance}$ Day * Time $F_{2.10} = 0.4775$, $p = 0.6217$; $ANOVA_{velocity}$ Day * Time $F_{2.11} = 4.631$, $p = 0.5367$]. Simple main effects revealed that time factor did have a statistically significant effect in total distance and mean velocity for both females (Fig. 2A, Fig. 2 B)[$ANOVA_{distance}$: $F_{2.10} = 22.81$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p < 0.0001$, d= 1.15; Day 1 vs Day 3 $p < 0.0001$, d= 1.23; Day 2 vs Day 3: $p = 0.997$, d= 0,12]; [$ANOVA_{velocity}$: $F_{2.11} = 35.59$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p < 0.0001$, d= 0.87; Day 1 vs Day 3 $p < 0.0001$, d= 1.16; Day 2 vs Day 3: $p = 0.9953$, d= 0.22] and males [$ANOVA_{distance}$: $F_{2.10} = 22.81$, $p = 0.0053$; multiple comparisons: Day 1 vs Day 2: $p = 0.0053$, d= 0.96; Day 1 vs Day 3 $p = 0.0002$, d= 1.24; Day 2 vs Day 3: $p = 0.5656$,

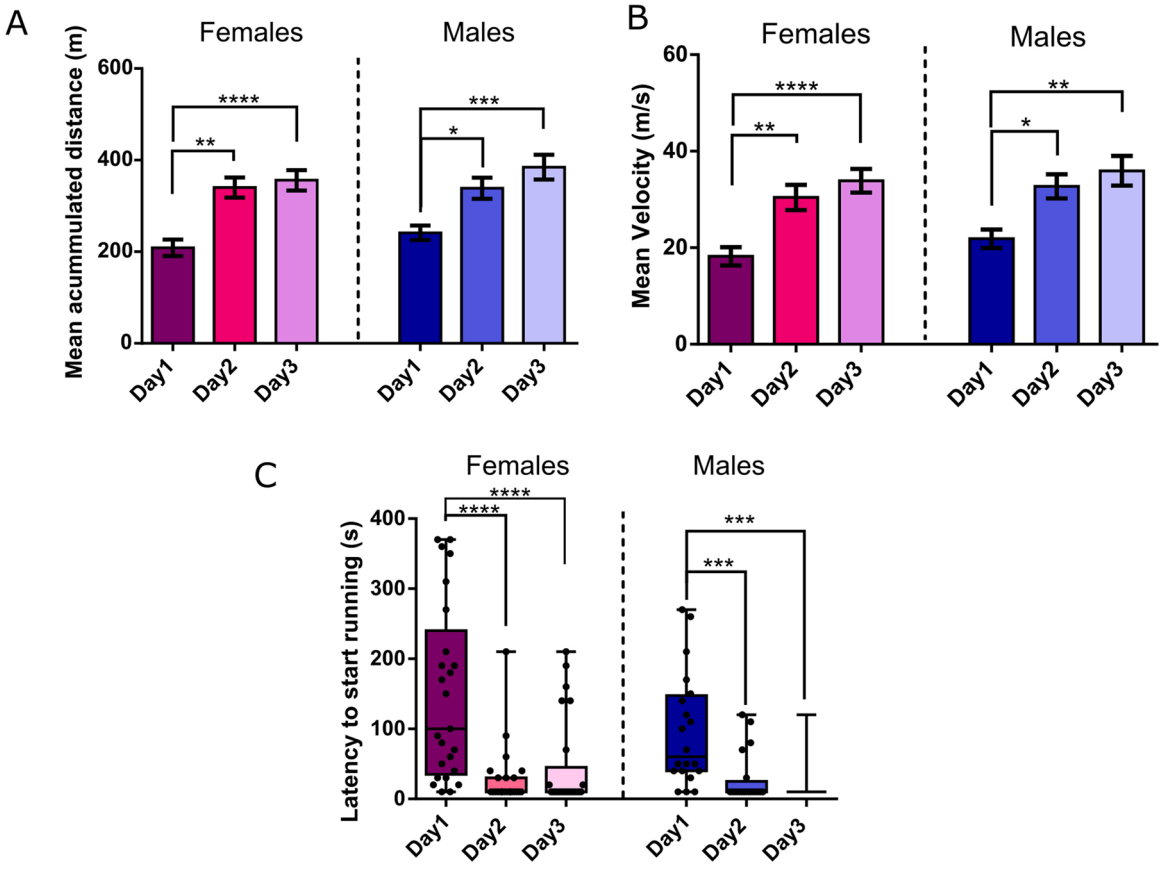

Fig. 2. A. Total accumulated distance traveled for female and male mice. Females: Day 1 vs Day 2: $p < 0.0001$; Day 1 vs Day 3 $p < 0.0001$; Day 2 vs Day 3: $p = 0.99$. Males: Day 1 vs Day 2: $p = 0.0053$; Day 1 vs Day 3 $p = 0.0002$; Day 2 vs Day 3: $p = 0.5656$. B. Mean accumulated velocity. Females: Day 1 vs Day 2: $p < 0.0001$; Day 1 vs Day 3 $p < 0.0001$; Day 2 vs Day 3: $p = 0.9953$. Males: Day 1 vs Day 2: $p = 0.0097$; Day 1 vs Day 3 $p = 0.0005$; Day 2 vs Day 3: $p = 0.6464$ C. Latency to start running. Females: Day 1 vs Day 2: $p = 0.0007$; Day 1 vs Day 3 $p = 0.0073$; Day 2 vs Day 3: $p > 0.999$. Males: Day 1 vs Day 2: $p = 0.0047$; Day 1 vs Day 3 $p = 0.0006$; Day 2 vs Day 3: $p > 0.999$.

d= 0.35], [ANOVA $_{velocity}$: $F_{2.11} = 35.59$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p = 0.0097$, d= 0.91; Day 1 vs Day 3 $p = 0.0005$, d= 1.02; Day 2 vs Day 3: $p = 0.6464$, d= 0.21], indicating an increase of those parameters between days 1 and 3 but not between days 2 and 3. In addition, latency to start running was analyzed. Significant differences were found between latency to run in day 1 and 2 for both males and females, but not between day 2 and 3 consistent with the results presented above (Fig. 2C) [$Q_{females} = 18.30$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p = 0.0007$; Day 1 vs Day 3 $p = 0.0073$; Day 2 vs Day 3: $p > 0.999$]; [$Q_{males} = 20.74$, $p < 0.0001$; multiple comparisons: Day 1 vs Day 2: $p = 0.0047$; Day 1 vs Day 3 $p = 0.0006$; Day 2 vs Day 3: $p > 0.999$]. No differences between males and females were found [$H_{females\ VS\ males} = 50.68$, $p < 0.0001$; multiple comparisons: Day 1 $_F$ vs Day 1 $_M$: $p > 0.999$; Day 2 $_F$ vs Day 2 $_M$: $p > 0.999$ Day 3 $_F$ vs Day 3 $_M$: $p = 0.5614$]. This last result indicates that once animals learn how to run on day 1, they quickly look forward to start running when exposed to the wheel in the subsequent days, as an important decrease in the latency to start running is observed. Thus, one day of wheel run is sufficient to trigger or encode a long-term running memory.

3.3. Taking breaks but running the same

Finally, we analyzed the number of breaks the animals take after they started to run as well as the total time animals spend off the wheel (Fig. 3 A, Fig. 3 B). No differences were found across days when evaluating the number of breaks and total break time [# Breaks: $Q_{females} = 3.714$, $p > 0.05$]; [$Q_{males} = 1.361$, $p > 0.05$]; [# Total Time Breaks: $Q_{females} = 2.102$, $p > 0.05$]; [$Q_{males} = 1241$, $p > 0.05$]. Moreover, negative significant correlations were found between the number of breaks/total time breaks over the total accumulated distance (Fig. 4). That is, the higher the number of breaks or breaks time, the lower the distance traveled. Notwithstanding, despite the number of breaks taken, animals travel longer distances on each day, suggesting that there is an increase in the number of revolutions performed across the days.

4. Discussion

In the present study we aimed to characterize the running pattern in CF1 mice as comparative wheel-running activity profiles of male and female in this strain remain unknown across literature. Data analysis showed a significant increase in daily running distance along days and velocity reaching a plateau in the final minute's animals spent running as no differences was found between day 2 and day 3 for both parameters, revealing that at least two days of experience with the running wheel are necessary to reach a maximum in distance traveled. It has been reported that mice typically run increasingly in the first days until a plateau level is reached (Fuss et al., 2010; Liebetanz et al., 2012; Bartling et al., 2017). However, those studies were carried out with other mice strains and with very different protocols as the large majority of studies applied voluntary exercise in running wheels for several weeks with free access to the wheel in their home-cage. Furthermore, they showed that most of the running was carried out in the active phase of mice behavior (night phase). In this sense, Bartling and co-authors, found that C57 mice engaged in a regular pattern of nocturnal running and, when analyzing recorded data, they observed that mice started running shortly after onset of the dark cycle/night phase (Bartling et al., 2017). Similar results were described by Malorni et.al where wheel-running activity was inhibited by light and enhanced by darkness in C57 mice. Moreover, this circadian running rhythm was in accordance with the animals' sleep pattern, where sleep was enhanced by light and inhibited by darkness (Oliverio and Malorni, 1979). Taking these results into account, wheel availability along the circadian cycle seems to play a key factor in the onset of the running behavior as, when given the opportunity, mice prefer to run in the active phase. Notwithstanding, in our work mice displayed a persistent running behavior pattern although experiments were conducted in the inactive phase of the cycle. It is worth pointing out that no sleep-like behavior was observed during our experimental protocols. Under this framework, the wheel itself not only could represent a novel object to explore but it also constitutes the only stimuli in the cage, in such a way that drives animals' motivation to start running.

On the other hand, our results did not reveal any differences regarding sex. Males and females showed the same pattern of running behavior for all the parameters analyzed. This result is in contrast with several reports that have found higher levels of running wheel activity in females than males (Koteja et al., 1999; Lightfoot et al., 2004; de Visser et al., 2007; Bartling et al., 2017). In this sense, it is worthy to consider the different factors that can affect running activity and consequently influence the outcomes of a voluntary running period. Relatively, little is known about the mechanisms mediating the differences in exercise capacity. Most studies compare animals of the same age, but no other parameters are taken into account such as differences in body weight and muscle mass in male and female animals of the same age. Moreover, as differences in running activity have been found in different strains (Lightfoot et al., 2004; de Visser et al., 2007), is not surprising to also found variability in terms of how sex influence physical activity in different strains. Thus, it is worth highlighting that this is the first study, to the best of our knowledge, to evaluate the running profile of both male and female CF-1 mice. In addition, no differences regarding sex-specificity were observed contributing to the variability in voluntary

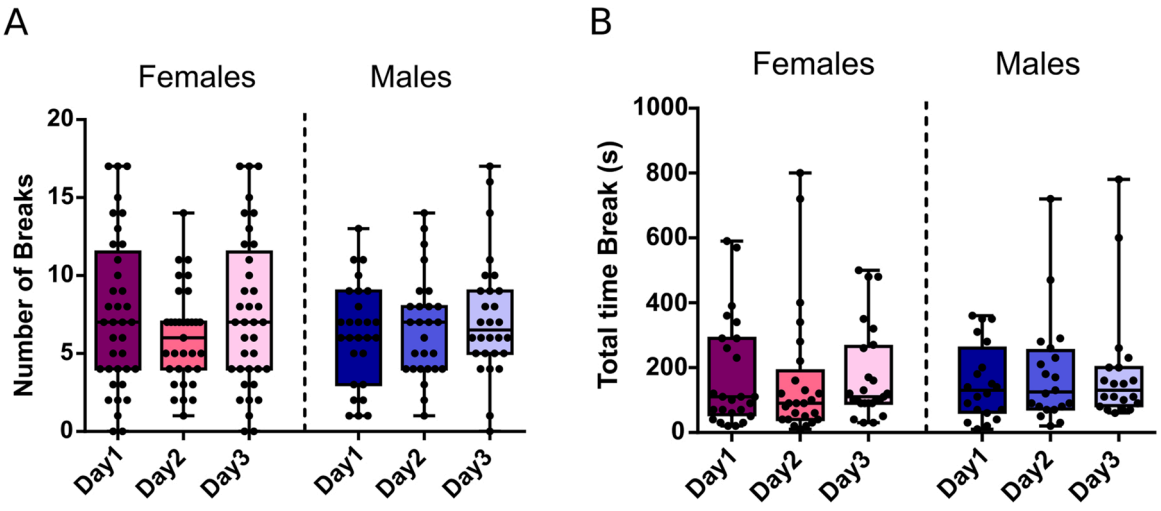

Fig. 3. A. Number of breaks. $p > 0.05$ B. Total time break. $p > 0.05$.

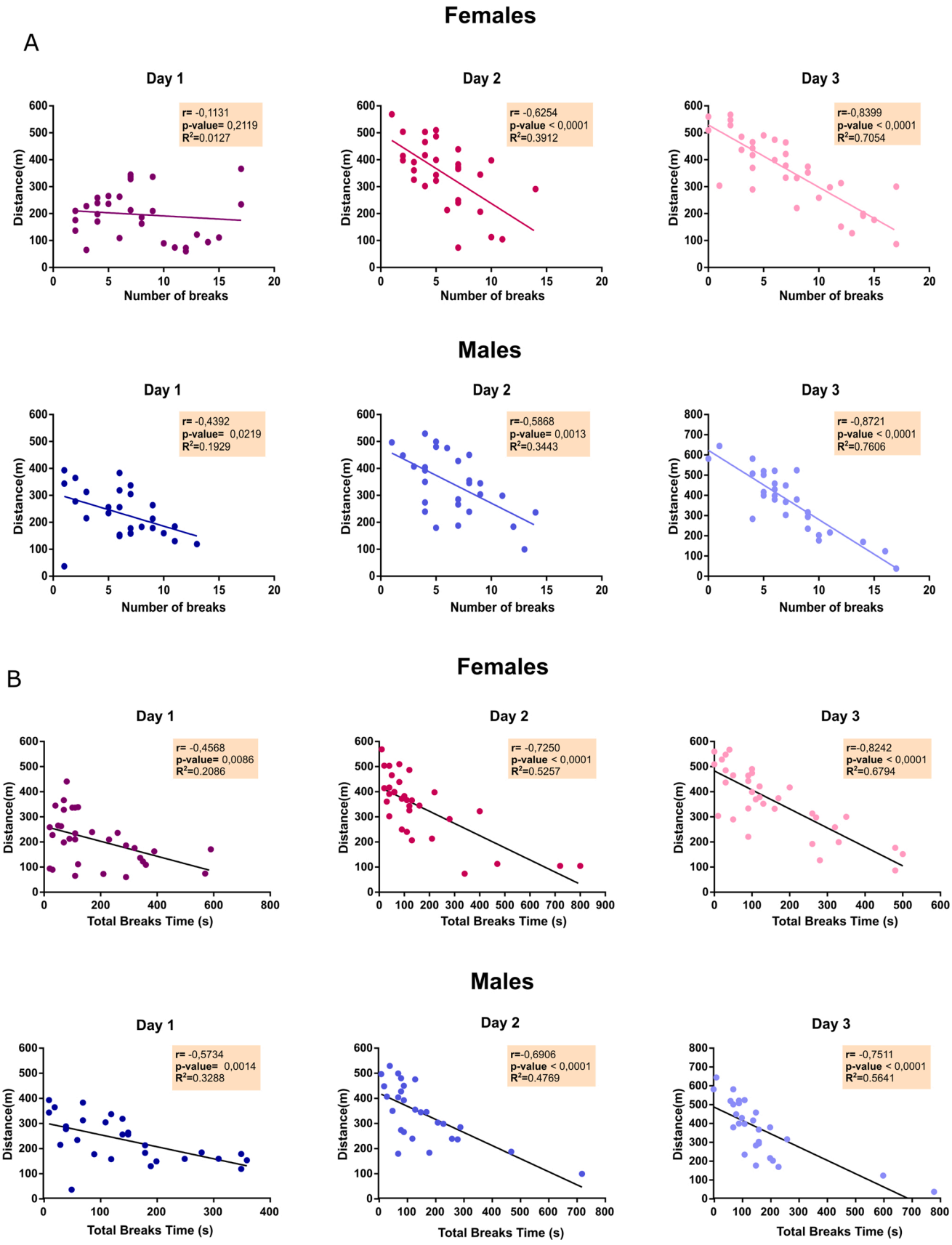

Fig. 4. A. Correlation between distance and number of breaks. B. Correlation between distance and total break time.

running activity.

In contrast to voluntary wheel running, forced activity is also used to study the effects of exercise in various topics such as learning and memory, aerobic endurance, mice models of osteoarthritis, gut microbiome among others (Koch and Britton, 2001; Ang et al., 2006; Kennard and Woodruff-Pak, 2012; Allen et al., 2015; Gronau et al., 2017). Forced activity approaches, such as treadmill running or swimming, have the advantage that animals can exercise at reproducible distances and speeds. However, the experimental conditions are usually non physiological and stressful; an aversive or unpleasant stimuli to induce exercise is used, such as an electric shock, touching the animals or the mere fear of drowning (Bernstein, 2003). This may lead to a pattern of activity that is far removed from normal mice behavior; reflecting behavioral, physiological and molecular responses with confounding effects, not strictly related to the exercise itself. Here, although the environment was restricted to the use of a wheel, no stimulus was used to force the running behavior. Thus, once animals learned to run, they voluntarily choose to repeat this behavior over others likewise exploratory activity. In this sense, some works argue about the nature of wheel running, claiming to be a stereotypic behavior promoted only in captivity (Sherwin, 1998; Mason et al., 2007; Mason and Rushen, 2008). Stereotypic behavior itself is characterized by several traits: it is repetitive, invariant and devoid of obvious goal or function (Mason and Latham, 2004). However, a recent study showed that wild mice run when a running wheel is available in nature and that bout lengths of running wheel behavior in the wild match those for captive mice (Meijer and Robbers, 2014). Why animals choose to voluntary perform this behavior is still an intriguing question, although existing explanations are that the rolling wheel is a consuming behavior that satisfies a motivation such as playing or escaping (Sherwin, 1998), or that it is linked to the metabolic system as a motor response to hunger or abroad stimuli related to foraging (Garland et al., 2011; Novak et al., 2012).

In addition to the analysis of distance traveled and velocity reached, we highlight the importance of evaluating other parameters such as the time the animals take to get on the wheel along the three consecutive days. Results revealed that animals that learnt how to run on day 1 quickly look forward to stepping into the wheel in subsequent training days, as a significant decrease in the latency to step into the wheel and begin running was observed. This result can be interpreted as the acquisition of two different types of memories, one coding for the hedonic valence of the learning event and the other for the procedural learning involved in the acquisition of motor skills. An intriguing question arises in terms of the mechanism involved in the run memory encode after only 20 min of wheel exposure. Costa and co-authors showed that one of the key brain regions involved in fast learning is the primary motor cortex (M1) where they found that fast motor skill learning was associated with substantial recruitment of neurons in M1 in behaving mice during the initial stages of learning an accelerating rotarod task (Costa et al., 2004) and with modulation of synaptic efficacy through long-term potentiation (LTP) and long-term depression (LTD) in rodents (Rioult-Pedotti et al., 2000). Further, the cellular mechanisms behind learning-related plasticity in M1 appear to depend on protein synthesis within this structure and specifically involve brain-derived neurotrophic factor (BDNF) (Kleim et al., 2003). In both humans and animal models, BDNF influences synaptic plasticity (Lu, 2003; Akaneya et al.). Injection of protein synthesis inhibitors targeting BDNF into the rat M1 induces a lasting loss of motor map representation (Kleim et al., 2003). Along these lines, targeting BDNF in the primary motor cortex after a bout of 20 min of wheel running could be an initial strategy to elucidate the mechanism implied in the encoding of this type of memory.

The hypothesis that animals learn how to run and remember to do it the next day falls within the framework that physical exercise is rewarding, thereby motivating animals to learn and to repeat the behavior. Various task attributes have a profound influence on long-term retention of skill learning. For instance, reward during practice improves long-term retention of a sequential motor skill (Abe et al., 2011). Evidence of the rewarding and potentially addictive properties of running wheels is currently accumulating (Belke and Wagner, 2005; de Visser et al., 2007; Greenwood et al., 2011; Novak et al., 2012). In mice, wheel running behavior is performed at the expense of other behaviors, such as resting and cage floor locomotion, and has the potential to disrupt the daily organization of activity (Harri et al., 1999; Visser et al., 2005). Behavioral studies investigating the rewarding properties of wheel running have unequivocally shown that rodents are highly motivated to gain access to running wheels and display conditioned place preference to an environment associated with wheel running (Lett et al., 2000, 2001; Trost and Hauber, 2014). In this sense the rewarding reinforcement of performing a behavior might be the driver in the formation of a long-term memory associated with exercise. This is in accordance with findings that exercising is related to neuronal plasticity, increasing neurogenesis and might be link between the improvements observed in other learning and memory process taken place after physical activity (Van Hoomissen et al., 2004; O'Callaghan et al., 2007; Gomes da Silva et al., 2012; Baruch et al., 2004; Burghardt et al., 2004, 2006; Van Hoomissen et al., 2004; Clark et al., 2008; Kohman et al., 2011).

On the other hand, a higher latency to step into the wheel on the first day could be interpreted in the framework of neophobia. In this sense, a run-wheel represents a novel object that in a first experience could drive an animal's fear towards it. Hence mice take longer times to start running until getting familiarized with the wheel. However, in line with what we have previously discussed, it is difficult to differentiate if the longer latency to step into the wheel on the first day is a consequence of animal neophobia expression or a procedural learning involved in the acquisition of motor skills which is attained over time. As far as we can tell, by the observations of mice behavior, animals have the tendency to explore the novel wheel stepping into it and instantly getting off of the wheel as they seem to get fear about the instability of the platform. After a few trials, mice get the ability to start running.

Altogether, the behavioral procedure described in this work is a starting point for the study of physical exercise as a modulator of cognitive processes. Most of the studies regarding mice performance on the running wheel focus on the larger effects of continuous exercise. Here we described a protocol that might serve to shed light into the acute effect of exercise in learning and memory task. In this sense, characterization of the running pattern in CF1 mice opens new venues to further exploring the effects of physical exercise on memory processes and extend them in psychopathology mice models.

5. Conclusion

Wheel running exercise is commonly used to study the effect of physical exercise in the prevention and improvement of pathophysiology of diseases to promote healthy aging as well as cognitive abilities. The patterns of voluntary wheel running vary between mice strains, age and sex. This variation must be accounted in the design of studies that aim to describe the beneficial effects of exercise and that may lead to the development of new strategies to enhance quality of life. In this work, we shed light on the running profile of CF1 males and female mice which, to the best of our knowledge, has not been described. This model can be particularly useful for long-term investigations exploring the effects of physical exercise on memory processes and extending them in psychopathology mice models.

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