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Research report

Genotype determining aerobic exercise capacity associates with behavioral plasticity in middle-aged rats

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ABSTRACT

Good aerobic fitness associates positively with cognitive performance and brain health and conversely, low aerobic fitness predisposes to neurodegenerative diseases. To study how genotype together with exercise, started at older age, affects brain and behavior, we utilized rats that differ in inherited aerobic fitness. Rats bred for Low Capacity for Running (LCR) are shown to display less synaptic plasticity and more inflammation in the hippocampus and perform worse than rats bred for a High Capacity for Running (HCR) in tasks requiring flexible cognition. Here we used middle-aged (~ 16 months) HCR and LCR rats to study how genotype and sex associate with anxiety and neural information filtering, termed sensory gating. Further, we assessed how inherited aerobic capacity associates with hippocampus-dependent learning, measured with contextual fear conditioning task. In females, we also investigated the effects of voluntary wheel running (5 weeks) on these characteristics. Our results indicate that independent of sex or voluntary running, HCR rats were more anxious in open-field tasks, exhibited lower sensory gating and learned more efficiently in contextual fear conditioning task than LCR rats. Voluntary running did not markedly affect innate behavior but slightly decreased the differences between female LCR and HCR rats in fear learning. In conclusion, inherited fitness seems to determine cognitive and behavioral traits independent of sex. Although the traits proved to be rather resistant to change at adult age, learning was slightly improved following exercise in LCR females, prone to obesity and poor fitness.

1. Introduction

Genotype is a known regulator of both brain structure (see for example [17,18,35] and overall level of cognitive function, also referred to as general intelligence (see for example [7,36,40]. Genetic loci explaining variability in the volume of subcortical brain structures such as putamen (motivation) and hippocampus (learning), seem to exist within genes involved in the development of neurons (i.e., size, growth of dendrites and axon, apoptosis) and their mutual interactions (i.e., synaptic stability and signaling) [17,18,35]. Further, it is widely accepted that genetics play a role in mental health, as for example, heritability of generalized anxiety disorder is approximated to be around 30 % [15] and several genes related to anxiety spectrum disorders have been identified [14,45].

In addition to governing the development of brain structure and function, genotype affects a feature extremely important for overall health, that is, innate aerobic fitness [37]. Aerobic fitness, on the other hand, not only promotes physical well-being but also associates positively with, for example, academic achievement in children [3] and lower anxiety and depression [20]. Further, physical activity is beneficial for both aerobic fitness and cognition [11]. Finding out the mechanisms of how innate and acquired aerobic fitness influence brain and

Abbreviations: CFC, contextual fear conditioning; HCR, high-capacity runner; LCR, low-capacity runner; OF, open field; PPI, pre-pulse inhibition; RW, running wheel.

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cognition might eventually help in devising effective and early interventions to promote brain health and well-being in the ever-growing population of inactive humans with low aerobic fitness.

To obtain this goal, we must start by determining the possible cognitive impairments brought about by innate and/or acquired low aerobic running capacity and the biological mechanisms responsible for the effects manifested at the behavioral level. For this, two rat lines originally developed through selective breeding, based on running capacity for studying metabolic diseases and aging (for a review see [24,25]) are extremely useful. These rats bred for either High or Low Capacity for Running (HCR and LCR, respectively) also differ in several other aspects. LCR rats exhibit overall lower physical activity [21,29] than HCR rats. Related to overall well-being, HCR rats are more responsive to stressors and possibly more prone to anxiety-like behavior than LCR rats [52]. LCR rats have lower mechanical sensitivity to pain [8], yet they exhibit enhanced sensation of pain compared to the HCR rats [28]. Regarding cognition, adult LCR rats exhibit impaired learning compared to the HCR rats [34,46,53], which might have to do with their more fragmented sleep [28]. Related, aged LCR rats have smaller hippocampi overall and a lower number of viable neurons in the CA1 than aged HCR rats [6]. Further, compared to HCR rats, LCR rats have reduced adult hippocampal neurogenesis already in adolescence [27], as well as increased inflammation both in the peripheral nervous system [8] and in the hippocampus [27].

To examine the connections between the genotype determining aerobic fitness, brain, and behavior, we subjected middle-aged, male and female, HCR and LCR rats to several behavioral tests. It is important to study the behavior on both sexes, as sex differences in prevalence of anxiety and depression are reported in both humans and rodents [2,30], and despite this, most studies are conducted with males only. In a separate group of animals, we also investigated whether a running intervention would abolish the possible genotype driven differences. We wanted to test if increased physical activity could modify behavior even in the middle-aged individuals, and on the other hand, whether the anxiety and startle responses remain the same as in young (two or ten months old) individuals [54]. In addition, we wanted to investigate the effect of human and/or other rats' presence on the behavior. Our hypothesis was that the genotype predisposing to poor aerobic fitness (LCR) would associate with impaired learning in both males and females, and that voluntary running would mitigate the differences between HCR and LCR rats. Finally, we hypothesized that voluntary exercise would reduce anxiety, especially in more anxiety-prone HCR rats.

2. Materials and methods

2.1. Ethical approval

All the experimental procedures were approved by the Animal Experiment Board of Finland (license ESAVI/12840/2019) and implemented in accordance with directive 2010/63/EU of the European Parliament and of the Council on the care and use of animals for research purposes. Throughout the study, special attention was paid to minimize all suffering, pain, and stress of the animals. Only experienced persons handled the animals.

2.2. Animals

In the current study, we used rats from lines selectively bred for high and low capacity for aerobic running [24]. Male and female rats from generation 42 and 43 of selection were bred and phenotyped, and then transported to Finland from The University of Toledo, Ohio, USA. The study was conducted in two parts, as experiment 1 and 2. All rats were housed at the Laboratory Center of the University of Jyväskylä in groups of two or three per cage (Makrolon IV, Techniplast, Italy) in experiment 1. For experiment 2, rats were housed individually in cages equipped with a running wheel. Aspen chips (Tapvei, Estonia) were used as bedding, nesting material and a plastic shelter were provided to the cages of the sedentary rats. Housing conditions were controlled with temperature at 21 ± 2 °C, and humidity at 50 ± 10 %. The rats were kept in a 12-hr light-dark cycle, with lights on from 8.00 a.m. to 8.00 p.m. Food (R36; Labfor, Lantmännen, Stockholm, Sweden) and tap water were available ad libitum.

2.2.1. Experiment 1: sedentary rats

The subjects (altogether 24 rats) were male and female retired breeders from the 42nd generation of HCR and LCR rat lines. The male and female rats were middle-aged, ~ 13 or ~ 15 months old, respectively, at the start of behavioral tests.

Female HCR rats in experiment 1 ($n = 5$, one rat excluded since it died from unknown cause) weighed 273 ± 8 g (mean ± standard error of mean) at the end of experiments while LCR females ($n = 6$) weighed 301 ± 9 g. Male HCR rats ($n = 6$) weighed 446 ± 19 g and LCR males ($n = 6$) weighed 510 ± 20 g in the end of behavioral tasks. Both rat line (Univariate ANOVA: $F[1,23] = 8.56$, $p = 0.009$, $\eta p2 = 0.311$) and sex ($F[1,23] = 146.21$, $p < 0.001$, $\eta p2 = 0.885$) had a significant main effect on body weight (interaction: $F[1,23] = 1.33$, $p = 0.263$, $\eta p2 = 0.066$). That is, LCR rats were heavier than HCR rats and males were heavier than females. The rats were tested for intrinsic maximal running capacity (a treadmill running test without prior training for running, see Koch et al. [24]) at the age of 12 weeks. The HCR rats ($n = 10$) ran on average 1957 ± 48 m whereas LCR rats ($n = 9$) ran 173 ± 11 m (One-way ANOVA $F[1,18] = 1194$, $p < 0.001$). There were no differences between the sexes in either of the rat lines, HCR males ($n = 4$) ran on average 1980 ± 77 m and females ($n = 6$) ran 1942 ± 66 m, whereas LCR males ($n = 5$) ran 159 ± 9 m and females ($n = 4$) 192 ± 21 m. Although a few of the rats were not phenotyped ($n = 5$), the HCR rats ran over 11 times further than LCR rats in the treadmill running test, showing clear difference in the maximal running capacity.

2.2.2. Experiment 2: voluntarily running rats

For experiment 2, altogether 14 HCR and LCR female rats from generation 43 were allowed to run voluntarily in running wheels (RW). For this experiment, the rats were housed individually in cages equipped with a running wheel (Ø 345 mm, Techniplast, Italy) for 6 weeks and then subjected to behavioral tests at approximately 17 months old. Voluntary running was recorded 24/7 with a recording system built in-house [21]. Custom-made software (Running Counter by Kimmo Lehti) was used to collect data of the wheel revolutions. The data were stored automatically once every second to a server (MS SQL-server 2014 Express). From the data, we analyzed the total distance (m) ran and circadian running behavior, using IBM SPSS Statistics 26 (IBM, Armonk, NY, USA). The circadian running behavior was calculated as an average from all days of the running intervention. The HCR RW females ($n = 7$) weighed 319 ± 17 g and the LCR RW females ($n = 7$) weighed 291 ± 6 g (see Fig. 1A), but there was no significant difference between the rat lines (ANOVA: $F[1,13] = 2.217$, $p = 0.162$). These rats were phenotyped for maximal running capacity at the age of 12 weeks. HCR rats ($n = 7$) ran on average 1641 ± 109 m and LCR rats ($n = 7$) 199 ± 25 m, the difference in the running distance being over 8-fold (one-way ANOVA: $F[1,13 = 167$, $p < 0.001$).

2.3. Behavioral tests

All rats were familiarized with handling and were weighed prior to the tests. Scoring of the behavior offline was done by a person naïve to the experimental groups.

2.3.1. Open field (OF)

The Open field (OF) test is a commonly used tool to measure rodent behavior, such as anxiety and locomotor activity [38]. All rats were exposed to an OF test twice. During the first OF exposure, the rats were

allowed to explore the arena made of white Plexiglass (floor: 76 cm × 76 cm, wall height: 40 cm) for 5 min while the experimenter stood outside the room. Standard ceiling light was provided, and the room was kept quiet. The arena was cleaned with 70 % ethanol between subjects to avoid smell contamination. The floor of the arena was lined with a black rubber mat to avoid reflections in the video. For the second exposure conducted a day later, the experimenter remained in the same room for the duration of the 5-min exposure. This was done so that the animals could get used to the presence of a human in the experiment room; for the social interaction test, a human presence would be necessary for safety.

All tests from above were recorded at 25 fps using a Basler ace (acA1440-220uc, Germany) camera. The videos were analyzed for the movement as well as time spent in the center of the arena by a person not familiar with the grouping of animals. For the analysis, a 4 × 4 grid was placed on top of the video and movement was quantified as the number of lines crossed. The center of the arena was defined as the area covered by the 4 center squares of the grid. Exploration was evaluated as the time spent in the center of arena. The animal was judged to be inside the center of the arena when its center of mass had crossed into the center. Further, the time spent grooming and the number of rearings were evaluated to characterize behavior in the open arena, as those reflect the animal's anxiety in a new environment and the willingness to explore the surroundings, respectively [26,39].

2.3.2. Social interaction test

The OF arena was used also for the social interaction test. During the 5-min test, one HCR and one LCR rat of the same sex and age were placed in the arena together. From the recorded videos, the latency to contact the other rat, number of initiated encounters and total time spent exploring the other rat were determined. The test was conducted as between-lines to better evaluate the differences of the rat lines, e.g. which rat line acts more dominant.

2.3.3. Pre-pulse inhibition (PPI)

PPI was used to study sensory gating [48]. PPI is a set-up where a weak stimulus (pre-pulse) is presented at varying intervals before a strong startle stimulus. It is thought that the processing of the pre-pulse stimulus inhibits or gates the generation of the startle response, hence the name of the test. Startle responses and PPI were tested using animal holder (ENV-264A, Med Associates Inc., Fairfax, VT, USA), which was placed to the platform (PH-250, Med Associates) in the sound insulated chamber (ENV-022S + speakers PHM-255A, Med Associates Inc., Fairfax, VT, USA). All rats were habituated to the chamber and the 70-dB background noise for 5 min twice (on separate days) before the actual recording. During the experiment, a 50-ms, 120-dB white-noise stimulus was used to elicit the startle response, and a 20-ms, 85-dB, 4-kHz tone as the pre-pulse stimulus. The experiment started with a 5-min stimulus free period after which 10 startle-alone trials were presented. The inter-trial interval varied between 10 and 20 s throughout the experiment. Next, 10 startle-alone trials and 40 PPI trials were presented in a pseudorandom order. During the PPI trials, the pre-pulse preceded the presentation of the startle noise by 30, 60, 100 or 200 ms, with an equal probability (10 trials each). At last, 10 more startle-alone trials were presented. After the last trial, the rat was returned to home cage.

Data from the startle and PPI were analyzed offline using Matlab (R2018b or newer, MathWorks, Natick, MA, USA). First, the maximum amplitude within 100 ms after the onset of the startle noise was derived from each trial and averaged across block (pre, during, post PPI) and trial type (startle alone, PPI 30 ms, 60 ms, 100 ms or 200 ms). Because body weight could affect the accelerometer-based measure, absolute values were proportioned to body weight (startle habituation analysis) or to the responses elicited during the first ten startle-alone trials (PPI).

2.3.4. Contextual fear conditioning (CFC)

During CFC, an unconditioned, aversive stimulus (electric shock) is paired with a neutral context (novel environment). The animal is expected to associate the context to an unpleasant stimulus and to react to the shock with a fear response (freezing). As the animal is placed to the same context on the following day, it is expected to retrieve the memory of the unpleasant shock and to then display freezing behavior [9,32].

The rats were placed in a standard rat conditioning chamber inside a quiet, dimly lit MDF-cabinet ((ENV-008CT and ENV-018MD, Med Associates Inc.). After 3 min of exploration, a single 1-s, 0.4-mA shock was delivered via a metal grid floor connected to a stimulator (ENV-414SA, Med Associates Inc.). The rat was then allowed to remain in the chamber for another minute and then returned to the home cage. The next day, ~ 20 h after the shock exposure, each rat was again placed in the same conditioning chamber for 3 min. During the fear conditioning and testing, the rat was monitored with a standard web-camera (Logitech) placed in front of the conditioning chamber. The video was stored at a rate of 30 fps for off-line evaluation of freezing behavior. Freezing was defined as the lack of all movement except that needed for respiration. Freezing was scored from the 3-minute training session (time prior to shock) and the 3-minute test session.

2.4. Statistical analyses

IBM SPSS Statistics 24 or 26 (IBM, Armonk, NY, USA) was used for statistical analyses. The data were analyzed using repeated measures (rm), univariate, and one-way ANalysis Of VAriance (ANOVA). Greenhouse-Geisser correction for p-values was used when the sphericity assumption was violated according to Mauchly's test. For the variables that were not normally distributed, either Mann-Whitney U or Kruskal-Wallis test was used. Pearson (r) or Spearman (r_s) correlation coefficient was used to detect linear correlation between two variables. For some variables in the first experiment the data of females and males was pooled together for statistical analysis, as differences between sexes were not significant. Because the runners and sedentary animals were from different generations of breeding and experiments were not simultaneously done, the groups were not compared with each other.

3. Results

3.1. HCR and LCR female rats ran similar distances in the running wheel voluntarily

In experiment 2, female HCR rats ran on average a total of 135.3 ± 55.5 km and female LCR rats 105.1 ± 32.5 km during the 37 days of intervention, making the daily distance on average 3 657 (± 1501) m and 2 842 (± 879) m, respectively (see Fig. 1C). There was no statistically significant difference between the groups in the total running distance ($U = 19$, $p = 0.775$). However, regarding the circadian rhythm, there was a statistically significant difference between the HCR and LCR rats in the daily running behavior (% of total daily distance/hour, calculated from total running across the intervention) around the time when lights were switched off, between 19.00 and 21.00 o'clock (see Fig. 1B, rm ANOVA: interaction of time and rat line: $F[1,10] = 11.84$, $p = 0.006$, $\eta p2 = 0.542$). Namely, LCR rats increased running in response to the onset of darkness compared to HCR rats.

3.2. HCR rats changed behavior in the Open-Field based on familiarity and/or the presence of a human experimenter, while LCR rats did not

To study the association of an inherited capacity for running with behavioral characteristics and learning in sedentary animals, six rats per line and sex (altogether 24 rats) underwent behavioral testing. In the OF sex had no effect on behavior. The HCR rats moved more in the OF during the first exposure, when left alone in the room, compared to the second session during which the experimenter stayed in the room (see Fig. 2A–C) (rm ANOVA: interaction of session and rat line: $F[1,20] = 10.16$, $p = 0.005$, $\eta p2 = 0.34$; effect of session in HCR rats: $F[1,11] =$

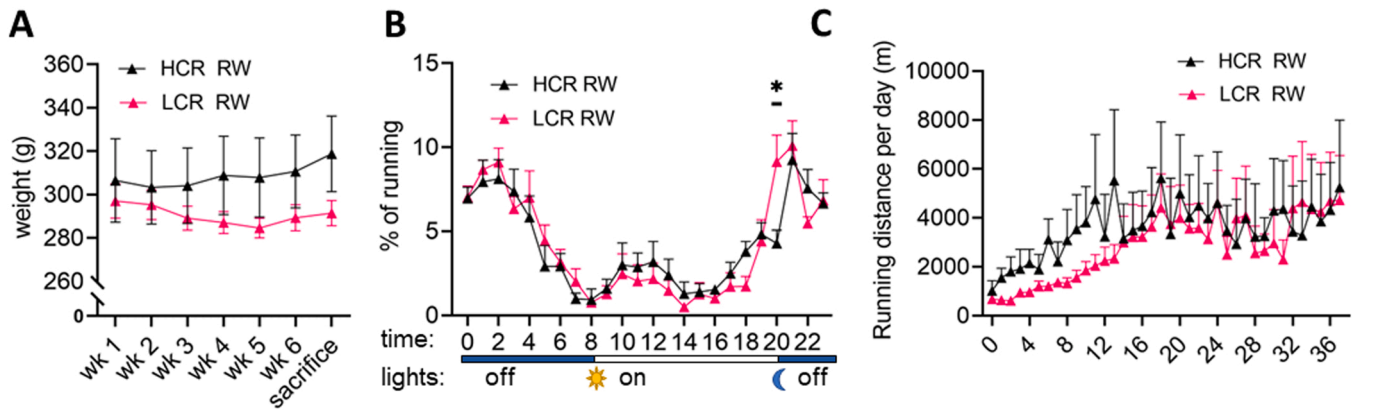

Fig. 1. Body weights (A), and timing of voluntary running (B) measured as an average across the 37-day period, and daily running distance (C) in female rats bred for a high capacity for running (HCR) or a low capacity for running (LCR). RW = running wheel. A) The weights of HCR and LCR rats remained stable throughout the 5.5-week running intervention. B) Running was more abundant in both rat lines during nighttime (lights off, 20.00–08.00), but the LCR rats increased running more than HCR rats after the lights were turned off. C) Both ratlines ran similar distances daily, no statistical differences were seen between the lines. Statistical significance of repeated measures ANOVA and one-way ANOVA is indicated with *, $n = 7$ per group. Asterisk refers to $p < 0.050$, error bars SEM.

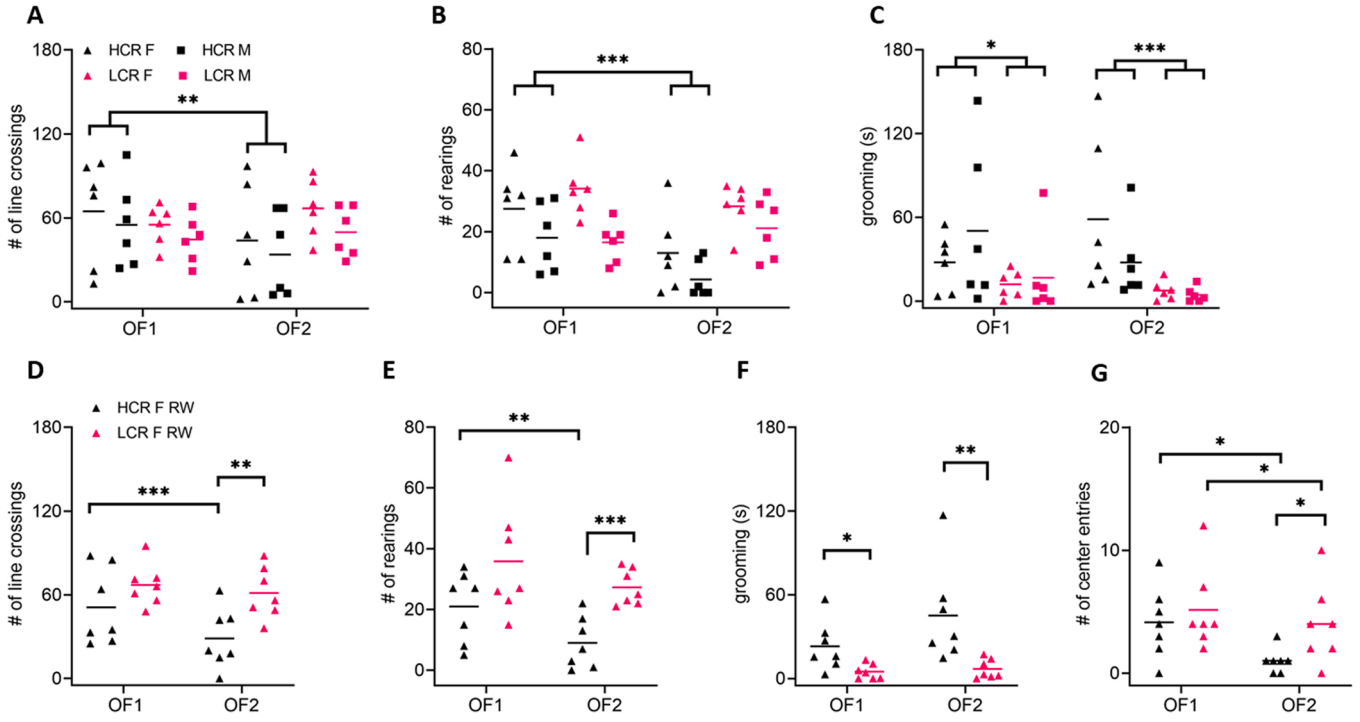

Fig. 2. Behavior in the open field (OF) arena revealed profound differences between rat lines bred for a high capacity for running (HCR) and those bred for a low capacity for running (LCR). Panels A–C: sedentary male and female HCR and LCR rats, panels D-G: female HCR and LCR rats that had access to a running wheel (RW). The data of males and females are shown separately to visualize the differences in behavior. Because the main effect of sex was not significant, for the statistical analyses data of males and females were pooled and visualized here with a connecting line. A) and D) HCR rats moved more (# of line crossings) in the open field arena during the first exposure (OF1, left alone) in comparison to second exposure (OF2, experimenter present in the room). There was no exposure-related difference in the behavior of LCR rats. B) and E) The number (#) of rearings decreased in the HCR rats but not in the LCR rats during the second exposure to the arena, when the experimenter remained inside the room. C) and F) HCR rats groomed (time, s) themselves more than the LCR rats, regardless of the presence of the experimenter during the second exposure. F) The LCR rats entered the center of the OF arena more often than HCR rats did (# of center entries). In all panels, statistical significances of repeated measures ANOVA and possible one-way ANOVA are indicated with asterisks: $p < 0.05$ (*), $p \leq 0.01$ (**) and $p < 0.001$ (***). In panels A–C, $n = 6$ per group, and in panels D–G, $n = 7$ per group. Sexes were pooled together for all statistical analysis and asterisks refer to differences between ratlines.

11.62, $p = 0.006$, $\eta p2 = 0.514$; mean line crossings in HCR vs. LCR, OF1: 60 vs. 50, 95 % CI [− 12.00, 32.00] and OF2: 39 vs. 58, 95 % CI [− 43.46, 4.46]). There was no effect of session/experimenter presence on movement in the OF in LCR rats (F [1,11] = 1.77, $p = 0.210$, $\eta p2 = 0.139$). The number of rearings decreased in the HCR rats but not in the LCR rats during the second exposure to the OF, when the experimenter remained inside the room (interaction of session and rat line: F [1,20] = 15.86, $p = 0.001$, $\eta p2 = 0.442$; effect of session in HCR rats: F [1,11] = 25.75, $p < 0.001$, $\eta p2 = 0.701$; in LCR rats: F [1,11] = 0.07, $p = 0.804$, $\eta p2 = 0.006$; mean HCR vs. LCR, OF1: 23 vs. 25, 95 % CI [− 13.21, 8.05] and OF2: 9 vs. 25, 95 % CI [− 24.59, − 7.58]). The HCR rats tended to groom themselves more than the LCR rats during

both sessions (main effect of rat line: F [1,20] = 10.90, p = 0.004, ηp2 = 0.353) (HCR vs. LCR, OF1: U = 38, p = 0.049 and OF2: U = 10, p < 0.001; mean HCR vs. LCR, OF1: 39 vs. 14, 95 % CI [− 3.84, 53.23] and OF2: 43 vs. 6, 95 % CI [9.94, 64.51]. To summarize, the HCR rats behaved differently in the OF arena based on whether the human experimenter stayed in the room or not.

The OF test was performed in the same way for the RW female rats (n = 6 for both rat lines, see Fig. 2D–G). There was no difference in movement between the rat lines when left alone in the room, but human presence decreased the movement of HCR rats (interaction of session and rat line: F [1,12] = 8.32, p = 0.014, ηp2 = 0.409; effect of session in HCR rats: F [1,6] = 33.46, p = 0.001, ηp2 = 0.848; mean HCR vs. LCR, OF1: 51 vs. 67, 95 % CI [− 41.76, 9.76] and OF2: 29 vs. 61, 95 % CI [− 55.83, − 9.31]). No significant effect of session was seen in the LCR rats (F [1,6] = 0.229, p = 0.229, ηp2 = 0.230). The number of entries to the center of the OF arena decreased in both rat lines, when a human was present in the same room (effect of session: F [1,12] = 7.58, p = 0.017, ηp2 = 0.387; mean HCR vs. LCR, OF1: 4 vs. 5, 95% CI [−4.68, 2.68] and OF2: 1 vs. 4, 95 % CI [− 5.81, − 0.19]) and LCR rats entered in the center more times than the HCR rats in the second session (U = 8, p = 0.038). Rat line or session did not affect the time spent in the center. Rearing decreased in the second session, especially in HCR rats (effect of session: F [1,12] = 13.07, p = 0.004, ηp2 = 0.521; effect of session in HCR rats: F [1,6] = 26.76, p = 0.002, ηp2 = 0.817; effect of session in LCR rats: F [1,6] = 2.72, p = 0.15, ηp2 = 0.312; mean HCR vs. LCR, OF1 = 21 vs. 36, 95 % CI [− 33.02, 3.31] and OF2, 9 vs. 27, 95 % CI [− 26.81, − 9.76]). Grooming increased overall in the second session (effect of session: F [1,12] = 7.756, p = 0.016, ηp2 = 0.393; mean HCR vs. LCR, OF1: 23 vs. 5, 95 % CI [2.83, 33.58] and OF2: 45 vs. 7, 95 % CI [8.59, 67.88]). HCR rats groomed more in both sessions than the LCR rats (OF1: U = 6, p = 0.017 and OF2: U = 1, p = 0.003). To summarize, the results of running female rats in the OF were in line with the sedentary counterparts. HCR rats behaved differently when a human was present, and overall displayed more signs of stress, e.g., by grooming themselves and moving less than the LCR rats. Thus, running did not counteract the effects of genotype on the OF behavior.

3.3. Sedentary and running LCR and HCR rats behaved similarly in the social interaction test

The next day after the second OF session, rats of the same sex but different line were let to explore the arena and each other in pairs. There were no differences in measures of social behavior (latency to contact the other rat, number of initiated encounters, and total time spent exploring the other rat) between the sexes or rat lines (mean HCR vs. LCR to contact 11 vs. 16 s, p = 0.347, and in contact: 57 vs. 67 s, p = 0.378 see Fig. 3A). In addition, rats of both sexes and rat lines moved about in the arena to a similar degree (statistics not reported, data not shown). The same trend was also seen in the running female rats in experiment 2; no rat line differences were detected in any of the behavioral measures (mean HCR vs. LCR to contact 12 vs. 12 s, p = 0.917, and in contact: 113 vs. 106 s, p = 0.702 see Fig. 3B). To summarize, the behavior of the male and female, HCR and LCR rats, independent of running, was similar in the social interaction test performed in the OF arena.

3.4. HCR rats react to startle more vigorously and Pre-Pulse Inhibition effects wear off faster than in LCR rats

Habituation to the startle-alone noise before, during and after (pre, during, post) PPI and the effect of the pre-pulse at different lead intervals (PPI 30 ms, 60 ms, 100 ms or 200 ms) were examined. The PPI at different lead intervals is reported as a percentage of startle reflex compared to the baseline that is startle alone trials. The amplitude of the startle response elicited prior to, during, and after the PPI-test differed between the HCR and LCR rats (rm ANOVA: main effect of rat line: F [1,20] = 8.36, p = 0.009, ηp2 = 0.295). Main effects of time or sex or their interactions were not statistically significant (F [2, 20/2, 40] = 0.25–3.49, p = 0.058–0.702, ηp2 = 0.149–0.066). That is, there were no differences between the sexes or rat lines in habituation to the startle stimulus, but HCR rats responded more vigorously to the startle noise than LCR rats.

PPI was similar in both HCR and LCR rats when the delay between the pre-pulse and the startle stimulus was 30 ms or 60 ms (one-way ANOVA: F [1,22] = 0.19/0.61, p = 0.667/0.442, respectively, mean HCR vs. LCR, 30 ms: 80 vs. 86 %, 60 ms: 54 vs. 46 %). However, when the delay was 100 ms or 200 ms, startle responses returned towards baseline in HCR but not in LCR rats (see Fig. 4B) (rm ANOVA, interaction of rat line and delay: F [3,60] = 6.32, p = 0.001, ηp2 = 0.240; one-way ANOVA: F [1,22] = 4.69/15.35, p = 0.042/0.001; mean HCR vs. LCR, 100 ms: 76 vs. 54 %, 95 % CI [0.92, 42.70] and 200 ms: 100 vs. 55 %, 95 % CI [21.60, 70.16]). To summarize, the PPI effect was equally large in both rat lines, but the effect wore off in the HCR rats faster than in the LCR rats. In LCR rats, the pre-pulse still inhibited the startle response when the delay was 200 ms.

For the running female rats, habituation to the startle-alone stimulus presented across the experiment (before, during and after PPI) was not statistically significant (F [2,24] = 3.06/1.33, p = 0.066/0.283). There was no main effect of pre-pulse lead interval or interaction of lead interval and rat line on the startle response (F [3,36] = 3.67/1.88, p = 0.055/0.185, ηp2 =0.234/0.135), but the main effect of rat line was statistically significant (F [1,12] = 6.76, p = 0.023, ηp2 = 0.360). The

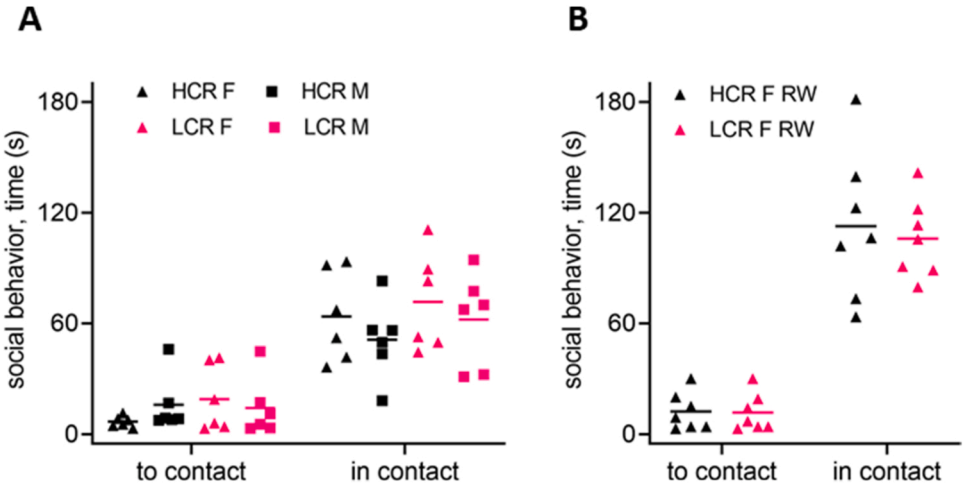

Fig. 3. Behavior in the social interaction test in A) sedentary male and female High and Low Capacity Runners (HCR and LCR, respectively) and B) females, that had access to a running wheel (RW). Social interaction skills were evaluated in the OF arena, where we determined the latency to approach another novel rat (to contact, s) and the total time spent in contact with that rat (in contact, s). Groups did not differ from each other, in panel A, $n = 6$ per group and in panel B $n = 7$ per group.

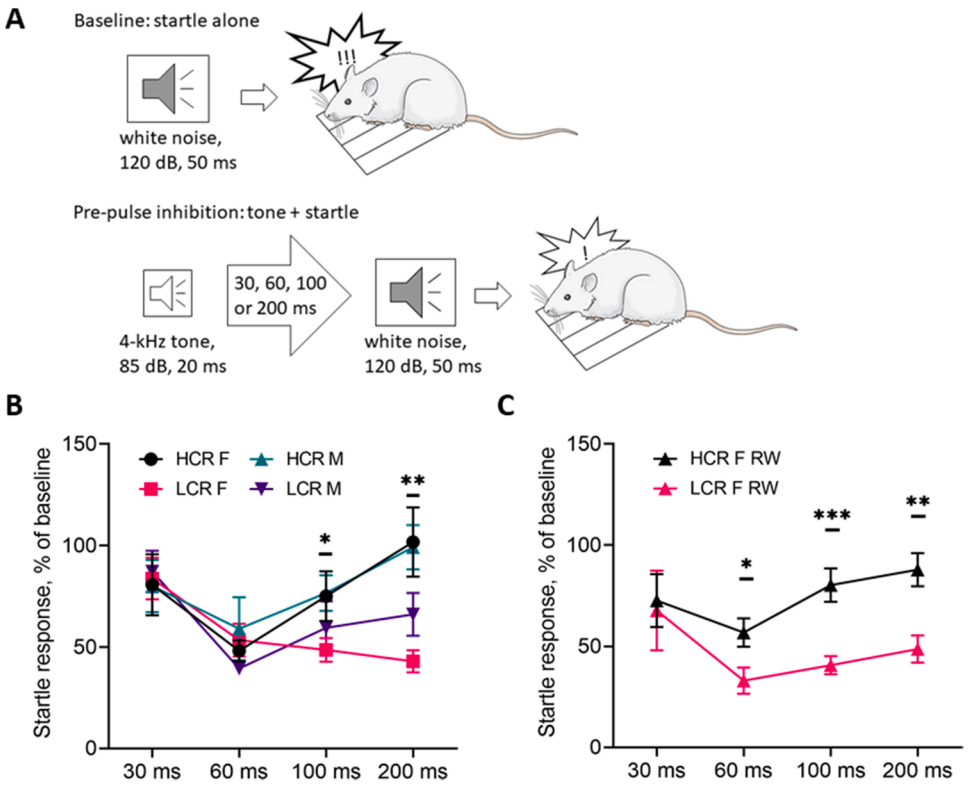

Fig. 4. Pre-pulse inhibition (PPI) differed between rats bred for a high capacity for running (HCR) and those bred for a low capacity for running (LCR). A) In the PPI experiment, rats were presented a white-noise startle stimulus alone or a prepulse tone and then the startle stimulus 30, 60, 100 or 200 ms later. B) PPI was elicited in both HCR and LCR rats (n = 6 in each group) to a similar degree at short delays (30 and 60 ms) while for the longer delays (100 and 200 ms) the effect was stronger in the LCR rats. The startle response was calculated as percentage relative to baseline (%) i.e., response to startle stimulus alone. Because the main effect of sex was insignificant, for the statistical analyses data of males and females were pooled. C) The PPI experiment was also conducted in female HCR and LCR rats that had access to a running wheel (RW). PPI was elicited in both HCR (n = 7) and LCR (n = 7) rats to a similar degree at the shortest delay (30 ms) while for the longer delays (60, 100 and 200 ms) the effect was stronger in the LCR rats. Statistical significances of repeated measures ANOVA and possible one-way ANOVA are indicated with asterisks: $p < 0.05$ (*), $p \leq 0.01$ (**) and $p < 0.001$ (***).

shortest lead interval (30 ms) between the pre-pulse and the startle stimulus resulted in equal startle responses in rats of both lines (F [1,13] = 0.042, p = 0.841, mean HCR vs. LCR: 73 vs. 68 %). However, there was a significant difference in the startle response between the rat lines at lead intervals of 60, 100 and 200 ms (see Fig. 4C) (F [1,13] = 6.12/17.39/13.56, p = 0.029/0.001/0.003; mean HCR vs. LCR, 60 ms: 57 vs. 33 %, 95 % CI [2.83, 44.72], 100 ms: 80 vs. 41 %, 95 % CI [18.86, 60.15] and 200 ms: 88 vs. 49 %, 95 % CI [16.00, 62.39]). To summarize, in running female HCR rats the PPI effect was smaller than in running female LCR rats, and the difference was already visible at a lead interval of 60 ms between the pre-pulse and the startle stimulus.

3.5. Context conditioned fear learning was more efficient in HCR rats than in LCR rats, independent of running

Rats of both lines showed minimal freezing in the novel environment, conditioning chamber during the first 3 min of the CFC training session, prior to the unpleasant electric shock (see Fig. 5), meaning that they were not initially afraid of the conditioning chamber. When placed in the same chamber 20 h later for the test session, HCR rats showed robust freezing, meaning that they were uncomfortable with the test chamber, connecting it to the electric shock given at training session, whereas LCR rats did not (rm ANOVA: interaction of rat line and session: F [1,20] = 22.40, p < 0.001, ηp2 = 0.528). Compared to the training session, the test session induced more freezing in both lines (HCR: F [1,11] = 38.93, p < 0.001, ηp2 = 0.780; LCR: F [1,11] = 7.82,

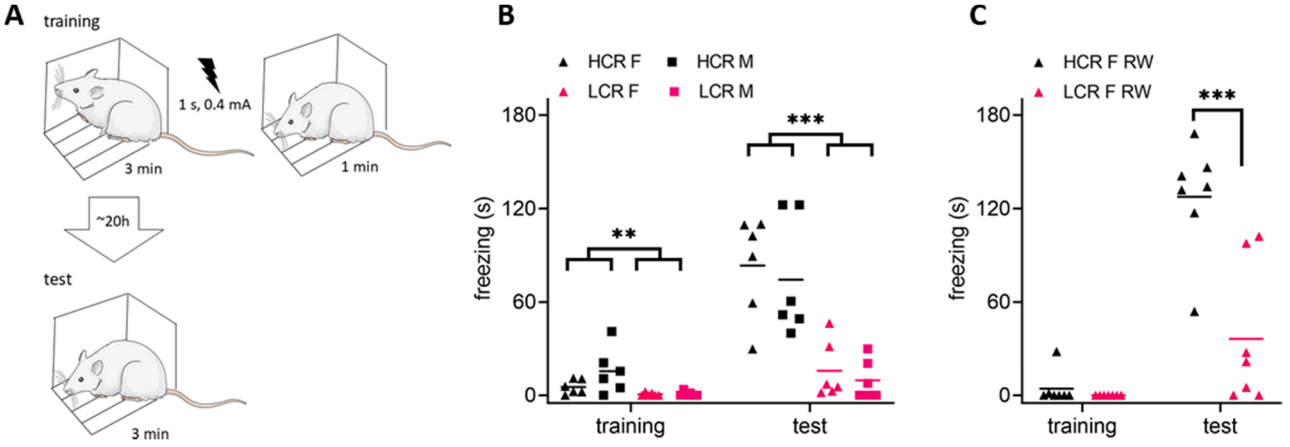

Fig. 5. Contextual fear conditioning was more efficient in HCR rats than in LCR rats, independent of running in a wheel (RW). A) Single-trial contextual fear conditioning was conducted, and learning was measured as freezing. Compared to LCR rats, fear learning was more efficient in both sedentary (B) and running (C) HCR rats. Statistical significance of repeated measures ANOVA and possible one-way ANOVA is indicated with asterisks referring to differences between rat lines, $p < 0.050$ (*), $p \leq 0.01$ (**) and $p < 0.001$ (***). In panel B, sexes were pooled for statistical testing. In panel B, $n = 6$ per group, and in panel C, $n = 7$ per group.

p = 0.017, ηp2 = 0.415), but HCR rats froze consistently more than the LCR rats (one-way ANOVA, training: F [1,22] = 8.24, p = 0.009, test: F [1,22] = 37.89, p < 0.001, mean HCR vs. LCR at training: 10 vs. 1 s, 95 % CI [2.28, 17.08], and at test: 79 vs. 13 s, 95 % CI [43.87, 88.45], respectively). Only one LCR rat froze for more than 36 s (= 20 % of time) during the test session, while 11 out of 12 HCR rats did so. There was no effect of sex on the freezing behavior. To conclude, HCR rats learned the CFC task better than LCR rats.

The running female rats in experiment 2 were subjected to CFC using the same protocol as for sedentary rats. The results were also very similar (see Fig. 5C, rm ANOVA, interaction of rat line and session: F [1,12] = 16.51, p = 0.002, ηp2 = 0.579). The HCR and LCR rats behaved similarly in the training phase, but in the test session HCR rats froze more than LCR rats (F [1,13] = 1.11/17.69, p = 0.313/0.001, respectively). The average time spent freezing for HCR vs. LCR during the training session was 4 vs. 0 s (95 % CI [− 5.58, 14.02]) and during the test 128 vs. 36 s (95 % CI [43.96 vs. 138.45]), respectively. To summarize, the HCR rats froze for about 70 % of the test session, which was over three times more than LCR. Thus, running did not fully counteract the effects of genotype on fear conditioning.

4. Discussion

We examined connections between the genotype determining aerobic capacity and behavior in male and female rats selectively bred for low or high running capacity. Our hypothesis was that low aerobic capacity would translate into lower capacity for cognitive tasks and behavioral plasticity. In accordance with our hypothesis, HCR rats outperformed LCR rats in hippocampus-based memory task, contextual fear conditioning. Voluntary running was expected to increase behavioral plasticity especially in rats with low inherited aerobic capacity, but we only saw a minor improvement in them, in the CFC task. However, regardless of voluntary exercise or sex, HCR rats were more anxious than LCR rats throughout our experiments, especially responding to the presence of the human experimenter. There was no difference in the voluntary wheel running (distance, m) in the middle-aged female HCR and LCR rats. However, voluntary wheel running behavior is different from general activity and especially from aerobic capacity, and thus can vary between animals and studies, although HCR rats generally tend to run more in running wheels than LCR rats do [31,43]. The results are discussed in more detail below.

4.1. Genotype predisposing to high aerobic capacity associates with more behaviors indicative of anxiety, independent of voluntary running

Our assumption was that high aerobic capacity would be beneficial for the individual especially in terms of neural and behavioral plasticity. However, this might not always be the case, as in our experiments, HCR rats displayed overall more signs of anxiety than LCR rats, especially in tests conducted in the open field arena. Behaviors related to anxiety in rats included more grooming, less locomotor activity (or more freezing) and decreased rearing. Grooming in a novel environment is considered as a sign of stress [39] whereas rearing is seen as an exploratory behavior, as it can help with information-gathering [26]. Locomotor activity and absence of freezing are also thought to be indicators of exploratory behavior and lack of anxiety [41]. These fear-related behaviors are thought to be analogous to anxiety seen in humans [30]. In our experiment, the behaviors indicative of anxiety increased in HCR rats, when a human was present in the OF test room. This finding might explain the underperforming of HCR rats in tests which are conducted in the presence of an experimenter [54]. Unfortunately, because of the low number of animals we could not perform the OF-tests in a pseudorandomized manner. Thus, as the experiment with a human presence was always second, we cannot fully rule out the possibility that LCR rats might just have habituated to the open field arena better than HCR rats. However, the fact that the HCR rats were even more anxious on the second exposure to the OF setting supports the idea that the human presence was the anxiety-increasing factor. Interestingly, in the social interaction test, rats independent of genotype were equally active and eager to seek contact to the other rat, although the experimenter stayed in the room, meaning that the anxiety in HCR does not extend to social context. Contrary to previously reported in humans and in several other rat lines and strains [2,30], we did not see any significant effect of sex on the features studied here.

Our results suggest that the HCR rats are more sensitive to environmental changes and are more cautious in new situations. In humans, previous studies have shown that exercise and good cardiorespiratory fitness protects against mental health disorders and anxiety [20,42]. In addition, personality traits of high extraversion and low neuroticism associate with more frequent participation and better performance in sports [1]. Neuroticism in general predicts lower daily physical activity in older adults [4], although trait anxiety i.e., tendency to feel anxious across many situations, might have a small positive association with daily light activity [4,22]. On the other hand, unusually high fitness level might predispose to higher anxiety, compared to average fitness [42]. In addition, exercise addiction is associated with higher trait anxiety and neuroticism [5]. Thus, it might be that the relationship between fitness and mental health follows an inverted U-curve [33], meaning here that both very low and very high levels of fitness could associate with compromised mental health, albeit likely for different reasons. As the selection process for aerobic capacity is based on the ability to perform in a forced treadmill test, it might select for animals with higher sensitivity and reactivity. Similar is seen in rat model bred for high and low response to training, where rats with high response to aerobic training are also more responsive to stress [49].

4.2. Genotype predisposing to high aerobic capacity associates with fast sensory processing and efficient learning with mixed effects of running

In addition to spontaneous behavior, we also studied the HCR and LCR rats in controlled environments and predicted that high fitness would link to better cognitive performance. First, we probed sensory gating within a PPI-paradigm. The inhibitory effect of a pre-pulse on the startle response was strongest at a 60-ms lead, as previously reported in other rodent studies [44]. Sedentary HCR rats displayed smaller PPI than LCR rats at lead intervals of 100 ms and above. This pattern of observations could be interpreted such that the auditory information processing in the HCR was faster. Research in humans has linked successful sensory inhibition to intelligence and better attentional control [19]. In contrast, in the running female rats the PPI effect was smaller in HCR rats than in the LCR rats even with a lead interval of 60 ms. That is, in the LCR rats sensory gating was more efficient than in the HCR rats. Thus, it seems that if running influenced sensory gating, in HCR rats it was worsened, which is at odds with human research: In humans, acute exercise increases PPI in anxious individuals [10]. Impaired sensory gating on the other hand is associated with multiple chronic brain disorders, most notably with schizophrenia [12] as well as anxiety [10]. To summarize, the present results regarding sensory gating in sedentary HCR and LCR rats echoes those seen in our previous studies [54]. Results regarding the effects of voluntary running, somewhat unexpectedly, also hint to the possibility that exercise might have a detrimental effect on sensory gating in the HCR rats. Due to the relatively small number of animals and the fact that the experiment was conducted solely on middle-aged female rats, the latter finding should be confirmed in further studies.

We also studied the effects of genotype predisposing to high vs. low running capacity on associative fear learning. We predicted HCR rats would learn better than LCR rats [53]. In line with our expectations, HCR rats learned to fear the context associated with a single foot-shock whereas LCR rats did not. To illustrate the magnitude of the difference, female HCR rats spent on average 46 % of the time in the test session frozen, whereas LCR females stayed frozen on average just 9 % of the

time. Fear conditioning relies on the hippocampus, amygdala, and the medial prefrontal cortex [23,47]. Hippocampus seems to be more critical for contextual fear conditioning, whereas the amygdala plays a bigger role in cued fear conditioning [32]. Of note, we recently reported lower levels of adult neurogenesis and higher expression of inflammatory markers in the hippocampi of LCR than HCR rats already at a young age [27]. Previous studies have linked pre-conditioning voluntary exercise to improved contextual learning [16,50]. Further, there is increasing evidence on the beneficial effect of exercise on hippocampal health overall, as studies have reported increases in neurogenesis, blood flow and growth factors, to name a few [51]. In line with these findings, in our current study, voluntary running improved contextual learning more in the LCR (by 100 %) than in the HCR (by 50 %) female rats, resulting in a diminished but still very clear difference between the rat lines (20 % vs. 70 % freezing in the test session, respectively). However, anxiety has previously been shown to correlate with better learning in fear conditioning task in rat models selectively bred for anxiety [13], meaning that anxiety might contribute to the superior learning of HCR rats. To conclude, in our study, the genotype predisposing to low aerobic running capacity [and poorer hippocampal health, [27]] associated with impaired learning but the impairment was slightly improved by voluntary running, even at middle-age.

4.3. Limitations

Because of limited resources, this study had a relatively small sample size, however, we were able to pool different sexes together for statistical analysis. For the same reason, we were able to conduct voluntary running intervention only in female rats. However, because these rats were from different generation of breeding, and the breeding might affect the selection of genes, we did not compare the data of runners and sedentary animals. In future studies, both sexes from same generations should be included, as the response to exercise might vary between the sexes.

5. Conclusion

This study demonstrates that the selection for the extremes of running capacity for over 40 rat generations results in phenotypes that differ also for behavior. Interestingly, we found HCR rats to be more anxious, but their ability to process information seems to be faster, and they learn better than LCR rats. That is, HCR rats seem to be more sensitive and faster, for both good and bad. Thus, behavior can reflect the inherited differences in aerobic fitness. Voluntary running did not affect the genotype-dependent differences in anxiety-related behavior but slightly improved contextual fear learning especially in the low-capacity runners. This study shows that exercise, even when started at an older age, might be beneficial for brain and cognition, especially in those at risk for obesity and metabolic disease. Further studies should be conducted to reveal the molecular mechanisms of the brain behind the effects reported here.

CRediT authorship contribution statement

E. Makinen: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **J. Wikgren:** Methodology, Software, Formal analysis, Data curation, Writing – review & editing. **S. Pekkala:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **L.G. Koch:** Methodology, Validation, Resources, Writing – review & editing, Funding acquisition. **S.L. Britton:** Methodology, Validation, Resources, Writing – review & editing, Funding acquisition. **M.S. Nokia:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **S. Lensu:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Data Availability

Data will be made available on request.

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Authors' contributions

MSN, SL, SP, and EM designed the study. MSN, EM and SL performed the experiment. MSN, EM, SL and JW analyzed the data. EM, SL, SP and MSN made the figures and wrote the manuscript. LGK and SLB developed the animal model used in this study. SL, MSN and SP acquired funding for the study. EM, SL and MSN wrote the draft. All authors have revised and accepted the final form of the manuscript.

Ethics approval and consent to participate

All the experimental procedures were approved by the Animal Experiment Board of Finland (license ESAVI/12840/2019) and implemented in accordance with directive 2010/63/EU of the European Parliament and of the Council on the care and use of animals for research purposes.

Consent for publication

N/a.

Competing interests

None.

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