Author(s): Wikgren, Jan; Nokia, Miriam S.; Mäkinen, Elina; Koch, Lauren G.; Britton, Steven L.; Kainulainen, Heikki; Lensu, Sanna

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Rats with elevated genetic risk for metabolic syndrome exhibit cognitive deficiencies when young

Jan Wikgren [a,*], Miriam S. Nokia [a], Elina Mäkinen [b], Lauren G. Koch [c], Steven L. Britton [d,e], Heikki Kainulainen [b], Sanna Lensu [a,b]

[a] Centre for Interdisciplinary Brain Research, Department of Psychology, University of Jyväskylä, P.O. Box 35, FI-40014 University of Jyväskylä, Finland
[b] Faculty of Sport and Health Sciences, University of Jyväskylä, P.O. Box 35, FI-40014 University of Jyväskylä, Finland
[c] Department of Physiology and Pharmacology, Center for Hypertension and Personalized Medicine, The University of Toledo College of Medicine & Life Sciences, Toledo, OH, 2801 W. Bancroft, Toledo OH 43606-3390, USA
[d] Department of Anesthesiology, University of Michigan, Ann Arbor, 1500 E Medical Center Drive, Ann Arbor, MI 48109-5048, USA
[e] Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, 7744 MS II, 1137 E, Catherine St., Ann Arbor, MI 48109-5622, USA

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ABSTRACT

Metabolic syndrome (MetS) is a known risk factor for cognitive decline. Using polygenic rat models selectively bred for high and low intrinsic exercise capacity and simultaneously modelling as low and high innate risk factor for MetS respectively, we have previously shown that adult animals with lower exercise capacity/higher MetS risk perform poorly in tasks requiring flexible cognition. However, it is not known whether these deficits in cognition are present already at young age. Also, it is unclear whether the high risk genome is related also to lower-level cognition, such as sensory gating measured as prepulse inhibition. In this study, young and adult (5-8 weeks and ~9 months) rats selectively bred for 36 generations as High-Capacity Runners (HCR) or Low-Capacity Runners (LCR) were tested for behavior in an open field task, modulation of startle reflex, and spatial learning in a T-maze. HCR rats were more active in the open field than LCR rats independent of age. Responses to the startle stimulus habituated to the same extent in LCR compared to HCR rats when young, but as adults, stronger habituation was seen in the HCR animals. The prepulse inhibition of startle response was equally strong in young HCR and LCR animals but the effect was shorter lasting in HCR animals. In T-maze, adult HCR animals unexpectedly showed attenuated learning, but we interpret this finding to stem from differences in motivation rather than learning ability. Overall, in the LCR rats with the risk genome for poor aerobic fitness and MetS, indications of compromised cognitive function are present already at a young age.

1. Introduction

Metabolic syndrome (MetS) is a health condition that increases the risk of non-communicable diseases such as conditions related to blood vessels and type II diabetes mellitus. Common characteristics of MetS include increased body fat, elevated blood pressure and blood glucose, high LDL cholesterol and plasma triglycerides. In addition to being a major risk factor for disease in general, MetS is also a significant contributing factor for cognitive decline [1]. It is assumed that impaired vascular reactivity, neuroinflammation, oxidative stress and abnormal brain lipid metabolism play a role [2]. Indeed, it has been found that adult individuals with MetS are more prone to ischemic stroke [3] and are more likely to have alteration in white matter [4] and brain metabolism [5]. Regarding adolescents, literature concerning the effects of MetS on neural function is relatively limited. However, even in adolescence, teenagers with presence of MetS factors, show volume losses in the hippocampus and frontal lobes [6], suggesting that conditions related to MetS might affect cognition already at younger age.

The interplay between MetS and physical activity is tightly coupled and affects cognition in opposing directions, but the relative contribution of each is more difficult to determine. Physically active lifestyle seems to protect from dementing conditions in older age [7, 8]. Using animal models, it has been shown that physical exercise, especially in an aerobic form, affects neural integrity for instance, by increasing the

* Corresponding author.
E-mail address: jan.h.wikgren@jyu.fi (J. Wikgren).

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amount of growth factors such as brain derived neurotrophic factor (BDNF) [9] and by facilitating hippocampal adult neurogenesis [10,11]. In humans, physical activity also increases the amount of white matter [12], which is linked to better learning [e.g., 13,14]. On the other hand, insufficient amounts of physical activity [15], a high-fat diet [16], and metabolic syndrome [2] have been linked to opposite observations. The beneficial outcomes of a physically active lifestyle are often attributed to aerobic fitness [17]. However, engagement in, and especially adherence to, physical activity is as much a result of personality traits [18] as it is the source of pleasure, social enrichment etc. All of them are known contributors to physical and mental well-being and cognition, rendering inferences of straight-forward causal relationships difficult.

Reducing the number of such confounding variables is possible by using selective breeding. As a result of selective breeding of rats based on intrinsic exercise capacity, Koch & Britton [19] have developed a heterogenic research model for energy transfer, representing also a model for MetS [20]. After 36 generations of selective breeding, the High-Capacity Runners (HCR) can run more than eight-fold longer on a treadmill in comparison with the Low-Capacity Runners (LCR) without any prior training. More importantly, the HCR rats are superior to the LCR rats in a number of health measures: e.g., HCR rats manifest less cardiac dysfunctions [21-23] and lack the symptoms of metabolic syndrome such as hypertension, visceral adipose tissue, and heightened blood lipid values [24,25]. Furthermore, the LCR rats show more neurodegeneration in hippocampus compared to HCR rats [26]. Studies show that the HCR rats are also more resilient to neural trauma induced by hemorrhage in the brain [27,28]. When tested for their cognitive abilities, the HCR rats outperform the LCR rats [26,29,30] and seem to be able to use more efficient and active coping strategies when exposed to stress [31]. Exercise is also shown to significantly activate various parts of the brain in HCR but not in LCR rats [32]. Finally, there are also differences in sleep, one of the well-established neuroprotective factors in favor for the HCR rats [33].

In sum, there seems to be several adaptive behaviors in which the HCR rats excel in comparison to the LCR rats. However, what is more elusive is whether the adverse effects related to a detrimental genome in LCR rats are already present at young age or whether they are parallel to the adverse health trajectory as a function of aging. To find out whether the detrimental genome for poor fitness and increased health risks affects factors relevant for cognitive abilities, we subjected young (~5-8 wks) and adult (~9 months) HCR and LCR rats (total N = 80) to a series of behavioral tests (open field activity, startle habituation, prepulse inhibition of startle response, and T-maze with rule reversal). We predicted that the HCR rats would be superior to the LCR rats in cognitive tests, and that these differences would become even clearer with age.

2. Methods

2.1. Ethical approval

All experimental procedures were implemented in accordance with the directive 2010/63/EU of the European Parliament and approved by the National Animal Experiment Board, Finland (ESAVI/7647/04.10.07/2014).

2.2. Animals

The rats used in this study were bred and housed on the premises of the animal research unit at the University of Jyväskylä. Food and water were freely available, and room temperature and humidity were controlled at 21 ± 2°C and 50% ± 10 percentage units, respectively. Rats had aspen chips (Tapvei, Harjumaa, Estonia) at the bottom of the cage as bedding material and plastic enrichment objects were placed in the cages. Rats were maintained on a 12/12 hour light/dark cycle, with lights on at 8 am. All experimental procedures were conducted during the light portion of the cycle.

The animals used in this study were 40 HCR and 40 LCR animals that were either ~5-8 weeks or 9 months old at the time of behavioral tasks. As the experiment required the use of young animals, we bred the offspring of generation 35 HCR/LCR animals that were previously shipped from the University of Michigan to the facilities of University of Jyväskylä. We used only male pups. At weaning (at the age of 3.7 wks) siblings were divided as equally as possible into both groups (young and adult). Rats were housed in groups of 2-3 per cage. As the difference between the lines has stabilized over generations and because we wanted to exclude any possible effects of running, the animals were not phenotyped for their exercise capacity. Among breeders in generation 35, the distance run to exhaustion was 2203.1 +/- 277 m for the HCR rats and 205.7 +/- 61 m for the LCR rats. The behavioral tests were conducted during the time course of three weeks starting when the young rats were on average 35 days and adults 234 days old. After the experiments, the animals were euthanized by a rising concentration of CO_2 and death was ensured by a cardiac puncture.

2.3. Open field activity

The open field arena was 75 × 75 cm square shaped area surrounded by white opaque Plexiglass walls of 40 cm in height. The arena was placed in an empty room with a recording webcam installed in the ceiling above it. The recording was started before the researcher placed the rat in the arena after which she went out of the room and closed the door. The duration of recording was 10 min and then number of droppings and urinations were counted before cleaning the arena with moist paper. Young rats were about 4.6 wks and adults 33.4 wks old during the test.

Behavioral analysis was performed offline by a trained evaluator blind to the purpose of the experiment or qualities of the animals. From the recordings, the evaluator was instructed to count the number of line crossings, latency to visit the central square, time spent in the central square, number of line crossings in the central square, number of rearing behaviors, and number of engagements in grooming behavior for the first 5 minutes of the recording.

2.4. Prepulse inhibition

The animals were tested two at a time in sound-insulated startle chambers (Med-Associates, Cambridge, UK) wherein the rat was placed in a cage, on a pressure-sensitive platform in front of speakers delivering the tones. The cage was small enough to prevent large movements. On average, the young rats were ~7.3 wks and adults were ~39 wks old when the prepulse inhibition task was conducted.

Prior to the experiment, the animals were accustomed to the chamber by placing them inside for 15 min with the background noise (~70 dB) on but no other stimuli were presented. The actual experimental protocol involved three blocks of trials; block 1: 10 startle alone trials, block 2: 10 startle alone trials + 40 trials with startle tone preceded by a prepulse tone with either 30, 60, 100, or 200 ms lead interval (in pseudo-random order), and block 3: 10 startle alone trials. The startle tone was a white noise of 50 ms in duration and 120 dB in intensity and the prepulse tone was 4000 Hz beep of 20 ms in duration and 85 dB in amplitude.

Maximum amplitude within 100 ms after the onset of the startle tone was derived from each trial and averaged across block and trial type. Habituation of the startle response was defined as the decrease of mean amplitude to the startle-tone alone trials from block 1 to 3. The PPI effect was calculated as relative amplitude of trials with a prepulse stimulus compared to startle alone trials within block 2. Weight difference might have a systemic effect on accelerometer-based measures. For this reason, we also calculated a relative measure for the habituation (how many percents the startle amplitude in block 3 was from startle amplitude in block 1).

2.5. T-maze protocol

The T-maze was placed in an empty room. The floor of the maze was made of brown film coated plywood and the side walls were dark brown acrylic of 17 cm in height. Each arm was 70 cm long and 10 cm wide. At both ends of the horizontal arms there was a 1-cm deep recess for the reward (rice cereal) to be placed in. The maze rested on three 90-cm high supports. From the maze, the animals had a full view on distal wall cues and some extra-maze clues such as plumbing, elements of air conditioning system and furniture. A web camera was placed on the ceiling above the maze and the rat's behavior was recorded for additional analyses afterwards. The animals were given some rice cereal beforehand to habituate them with the reward. Rats were familiarized with the T-maze for three days in altogether 6 pretraining sessions where both recesses were baited and the rats were free to explore the maze for 2 min at the time, 5 times in each session. In the following three days, two rats were simultaneously trained to seek the reward in only one direction by forcing the rat to turn eight times right and eight times left in random order, while the entry to the other arm was blocked. There was at least a 30 seconds intertrial interval between maze visits for the same individual. The experimenter closed the gate of the baited arm after the rat was eating the cereal and gently took the rat back to its homecage. Maximum time for each maze visit was two minutes.

The actual training phase took place on the fourth day, when young rats were ~6.8 wks and adults were 37 wks old. Both arms were open and for each individual rat, either the left or right arm was consistently baited. The discrimination training consisted of five repeated blocks, each block having 12 trials with at least 30 seconds intertrial interval between them. The rat was considered to have made a correct decision, when whole animal (including the tail) had entered the arm, after which the arm was closed. Reversal training took place on the same day after about two hours hiatus. In reversal training, the arm opposite to the arm in the discrimination training phase was baited and four blocks of 12 trials were performed. The number of correct choices out of 12 possible was used as the outcome in statistical analyses.

2.6. Statistical analyses

SPSS software was used in all statistical analyses. The specific methods and factor models are described in the results.

3. Results

3.1. Body weight

At weaning rats of both lines weighed the sa.me (HCR 68.4 ± 10 g [mean ± standard deviation] vs. LCR 69.3 ± 8 g). At the time of the open field tests, young rats of both lines still weighed the same: HCR rats 94 ± 18 g and LCR rats 92 ± 11 g. At the time of the startle experiments, after 7 weeks of age, the weight differed between the rat lines, average weight of young HCR rats being 154 ± 24 g and that of the LCR rats 185 ± 61 g. In the adult group, the average weight during behavioral tasks was 374 ± 43 g in HCR rats and 467 ± 88 g in LCR rats. Independent samples t-test confirmed that the differences were significant for both young [t (37) = 2.17; $p < 0.05$; d = 0.67] and adult animals [t (37) = 4.21; $p < 0.001$; d = 1.34].

3.2. Open field activity

Measures of locomotion, exploratory behavior and signs of anxiety were assessed from the open field videos off-line and examined for differences using univariate ANOVA with line and age group as fixed factors. The results and statistical values are presented in detail in Figure 1 and Table 1, respectively.

Overall line crossings were more frequent in HCR rats [F(1,173) = 23.18; $p < .001$; partial $\eta^2 = 0.241$] and in young animals [F(1,173) = 263.20; $p < .001$; partial $\eta^2 = 0.783$]. There were no significant effects

Table 1
ANOVA for open field activity variables

		df	F	p	η^2 p
Line crossings	line	1,73	23.183	**< .001**	0.064
	age	1,73	263.198	**< .001**	0.730
	line X age	1,73	1.248	.268	0.003
Latency to middle	line	1,73	0.038	.845	0.001
	age	1,73	1.574	.214	0.021
	line X age	1,73	0.313	.577	0.004
# middle visits	line	1,73	7.831	**.007**	0.097
	age	1,73	19.174	**< .001**	0.208
	line X age	1,73	0.000	.993	0.000
Time middle	line	1,73	0.108	.743	0.001
	age	1,73	0.758	.387	0.010
	line X age	1,73	0.492	.485	0.007
Rearing	line	1,73	25.778	**< .001**	0.261
	age	1,73	5.725	**.019**	0.073
	line X age	1,73	12.542	**< .001**	0.147
Grooming	line	1,73	0.863	.356	0.012
	age	1,73	0.388	.535	0.005
	line X age	1,73	1.312	.256	0.018
Droppings	line	1,73	3.339	.072	0.043
	age	1,73	0.619	.434	0.008
	line X age	1,73	2.001	.161	0.026
Urination	line	1,73	0.056	.814	0.001
	age	1,73	5.594	**.021**	0.070
	line X age	1,73	0.004	.952	0.000

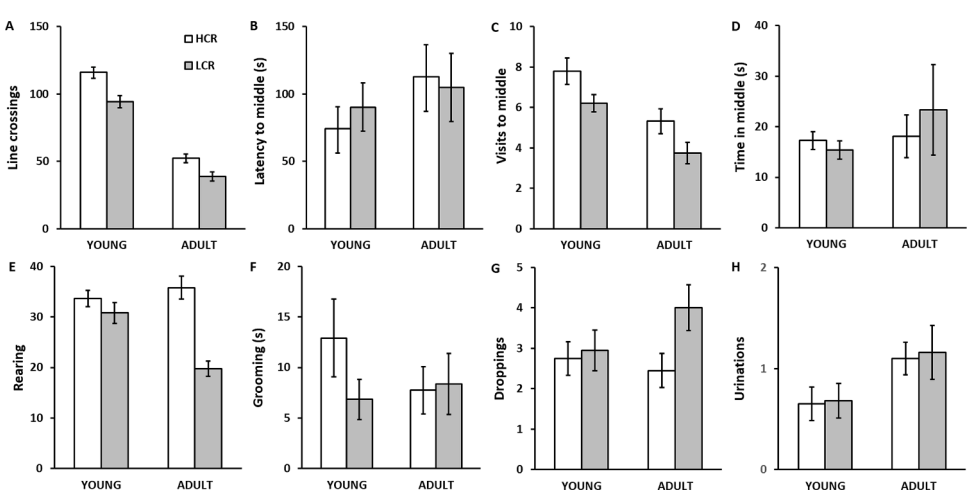

Figure 1. Open field activity results. Bars represent means (+/- SEM)

concerning the latency to visit the middle area, but in the number of visits to the middle area, significant main effects of line [$F(1,173) = 7.83$; $p < .01$; partial $\eta^2 = 0.09$] and age [$F(1,173) = 19.17$; $p < .001$; partial $\eta^2 = 0.208$] were found. This was because young animals visited the middle area more often than adult animals and because HCR rats did so more often than LCR rats. Concerning the time spent in the middle or number of feces dropped on the arena, no significant effects were found.

In rearing behavior, there was a significant line X age interaction [$F(1,173) = 12.54$; $p < .01$; partial $\eta^2 = 0.147$]. Follow-up t-tests indicated that this was due to less frequent rearing behavior in the adult LCR rats compared to adult HCR rats [$t(36) = 5.96$; $p < .001$; $d = 1.93$] and decline in rearing behavior as a function of age in LCR rats only [$t(36) = 4.38$; $p < .001$; $d = 1.42$]. Concerning grooming behavior, no significant effects of line or age were found.

In sum, HCR animals tended to be more on the move in the open field arena and as a function of age, activity declined. Rearing behavior decreased in the LCR rats as a function of age but not in the HCR rats.

3.3. Startle habituation

Repeated measures ANOVA, with line and age as between subjects and trial block as a within-subjects factor, was performed on startle response amplitudes (Figure 2). There was a significant main effect of trial block [$F(2,148) = 41.42$; $p < .001$; partial $\eta^2 = 0.36$], indicating that the response diminished as a function of time, and age [$F(1,74) = 40.98$; $p < .001$; partial $\eta^2 = 0.36$], indicating overall larger responses in adult animals. The trial block X line interaction approached significance [$F(2,148) = 2.45$; $p = .09$; partial $\eta^2 = 0.03$].

In a separate ANOVA for young animals, the main effect of block was significant [$F(2,74) = 16.25$; $p < .001$; partial $\eta^2 = 0.31$]. Neither the main effect of line [$F(1,37) = 0.74$; $p = 0.110$; partial $\eta^2 = 0.06$] nor the trial block X line interaction [$F(2,74) = 0.74$; $p = 0.110$; partial $\eta^2 < 0.01$] was significant indicating that habituation occurred at equal pace in both groups. Non-significant independent samples t-test result for relative measures of startle habituation [$t(37) = 0.36$; $p = 0.720$; $d = 0.11$] also indicated that the startle response had diminished roughly equally in both groups.

For adult rats, the main effect of trial block was significant [$F(2,74) = 25.90$; $p < 0.001$; partial $\eta^2 = 0.41$] indicating habituation over time. Main effect of line was not significant [$F(1,37) = 0.36$, $p = 0.55$; partial $\eta^2 = 0.01$], but the line X trial block interaction [$F(2,74) = 5.11$; $p < 0.01$] indicated stronger habituation in the HCR animals. However, the difference in relative startle habituation (Figure 3C) did not quite reach significance [$t(37) = 1.68$, $p = 0.1$, $d = 0.54$], even though the effect size indicated somewhat meaningful difference.

Thus, in young animals, there were no differences in startle habituation, but in adults, the rate of habituation was stronger in HCR.

3.4. Prepulse Inhibition

The effect of presenting a brief prepulse stimulus with different lead intervals prior to the startle stimulus (PPI effect) on the startle response is depicted in Figure 3. As seen, the 20 ms lead interval led to increased startle amplitude, most likely indicating that the animals were not able to perceive the prepulse and the startle stimulus separately. Thus, we decided to exclude this interval from further analyses. In repeated measures ANOVA with line and age as between-subjects variables and lead interval as within-subjects variables, there were significant main effects of lead interval [$F(2,148) = 59.54$; $p < .001$; partial $\eta^2 = 0.446$] and line [$F(1,74) = 8.35$; $p < .01$; partial $\eta^2 = 0.101$], whereas main effect of age was not significant [$F(1,74) = 0.87$; $p = .353$; partial $\eta^2 = 0.012$]. Lead interval X line interaction was also significant [$F(2,148) = 19.73$; $p < .001$; partial $\eta^2 = 0.210$] and lead interval X age interaction nearly so [$F(2,148) = 3.02$; $p = .056$; partial $\eta^2 = 0.039$]. Lead interval X line X age interaction was not significant [$F(2,148) = 0.14$; $p = .870$; partial $\eta^2 = 0.002$].

Separate univariate analyses of variance for each lead interval were then conducted. With all intervals, there was a significant or nearly significant main effect of line [for 50 ms: $F(1,74) = 3.18$; $p = .079$; partial $\eta^2 = 0.041$, for 100 ms: $F(1,74) = 14.23$; $p < 0.001$; partial $\eta^2 = 0.161$, and for 200 ms: $F(1,74) = 17.56$; $p < .001$; partial $\eta^2 = 0.041$]. Neither the main effects of age nor age X line interactions were significant.

Finally, one sample t-tests were conducted on the responses in each subgroup at each interval against the baseline value (100%). In young animals, the HCR deviated from baseline at 50 ms intervals [$t(19) =$

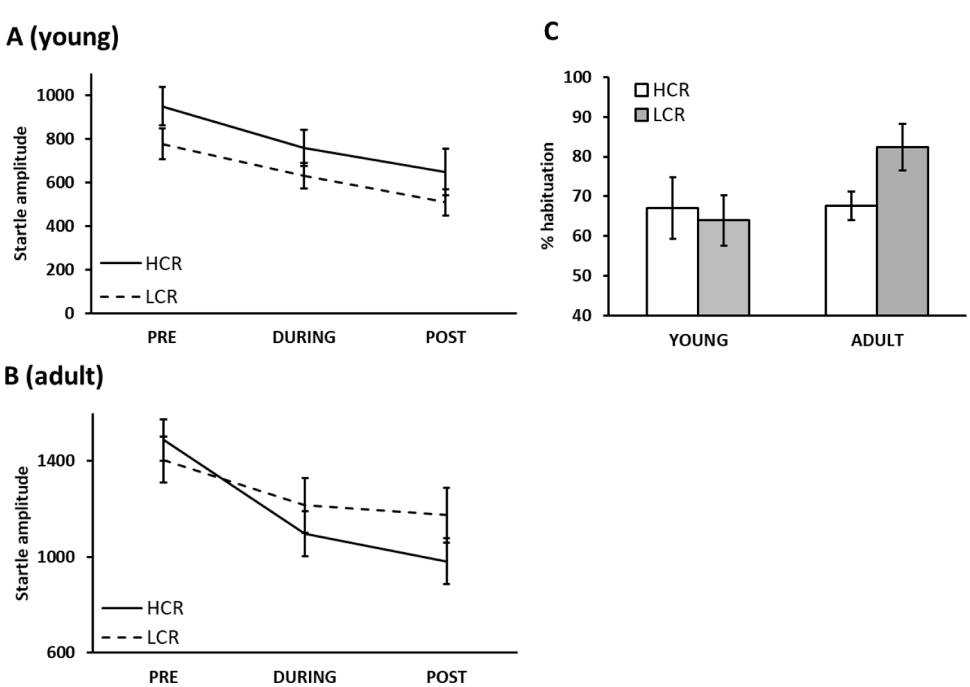

Figure 2. Habituation of the startle response (absolute values) in young (A) and adult (B) animals. C. Relative decrease in mean startle amplitude. All values represent means (+/- SEM)

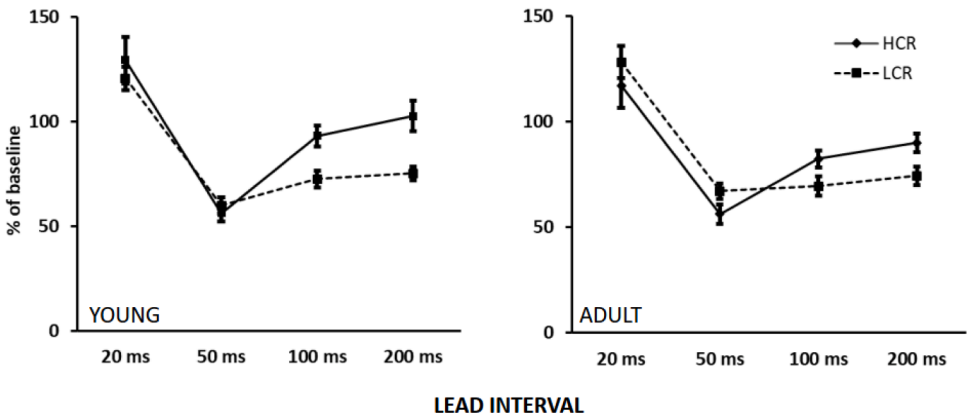

Figure 3. Prepulse inhibition effect in young and adult animals. The values represent mean per cents (+/- SEM) in relation to response amplitude in startle alone –trials.

11.23; $p < .001$, $d = 2.51$] but not at longer intervals whereas the LCR deviated from baseline at all intervals [$t(19) = 6.96 - 10.10$; $p < .001$, $d = 1.60 - 2.31$]. In adult rats, all tested differences were significant [HCR: $t(16) = 2.19 - 7.31$; $p < .001 - .05$; $d = 0.55 - 1.83$, LCR: $t(15) = 9.32$ – $p < 0.001$; $d = 1.33$ – 2.05]

The results demonstrate that at young age the PPI effect was as large in both groups, but the HCR might be faster to process information as their startle responses return to normal already at 100 ms. In adults, the same pattern can be seen but even the HCR do not quite reach the baseline level startle response level, even when the longest, 200-ms, lead interval is used.

3.5. T-maze

Repeated measures ANOVA, with trial block as a within-subjects variable and line and age as between-subjects variables, was conducted for the number of correct choices separately. This was done for the discrimination and reversal training phases for all animals that completed the planned number of trial blocks.

Results are depicted in Figure 4. In repeated measures ANOVA concerning the discrimination phase, there were significant main effects of trial block [$F(4,224) = 39.51$; $p < .001$; partial $\eta^2 = 0.414$], line [$F(1,56) = 9.65$; $p < .01$; partial $\eta 2 = 0.15$] and age [$F(1,56) = 5.42$; $p < .05$; partial $\eta 2 = 0.09$]. Line X age interaction was also significant [$F(1,56) = 4.80$; $p < .05$; partial $\eta^2 = 0.08$]. Taken together, these results indicate that there was no difference in the rate of learning between groups but the overall the number of correct trials remained relatively low in the adult HCR group.

Similar analysis regarding the reversal phase yielded significant trial block X line X age interaction [$F(3,168) = 2.97$; $p < .05$; partial $\eta 2 = 0.05$] as well as significant main effects of block [$F(3,168) = 191.48$; $p < .001$; partial $\eta 2 = 0.77$] and age [$F(1,56) = 5.12$; $p < .05$; partial $\eta 2 = 0.08$]. In the subsequent 2 (line) X 4 (trial block) repeated measures ANOVA for both age groups separately, revealed significant line X trial block interaction in young [$F(3,111) = 3.77$; $p < .05$; partial $\eta 2 = 0.09$] but not in adult animals [$F(3,57) = 0.65$; $p = .585$; partial $\eta 2 = 0.03$]. These results indicate that as a function of age, there is a drop in HCR rats' performance in the reversal learning.

4. Discussion

We studied behavioral tendencies, sensory adaptation, and learning in young and adult rats bred for high (HCR rats) or low (LCR) intrinsic running capacity which also models for an innate risk for metabolic syndrome (low vs. high risk, respectively) [20]. Of the polygenic rat lines, the fit and healthy HCR rats were more active and curious, tended to adapt faster to their surroundings, and their auditory information processing was more efficient. Results regarding spatial learning with rule reversal remained somewhat unclear. The differences between LCR and HCR rats were mostly already present at young age, suggesting that the detrimental genome making an individual prone to MetS may affect brain and cognition already at an early age. Although the difference in body weight developed within about seven weeks and was not as large at a young age as it was in adult rats, the LCR rats differed from HCR in

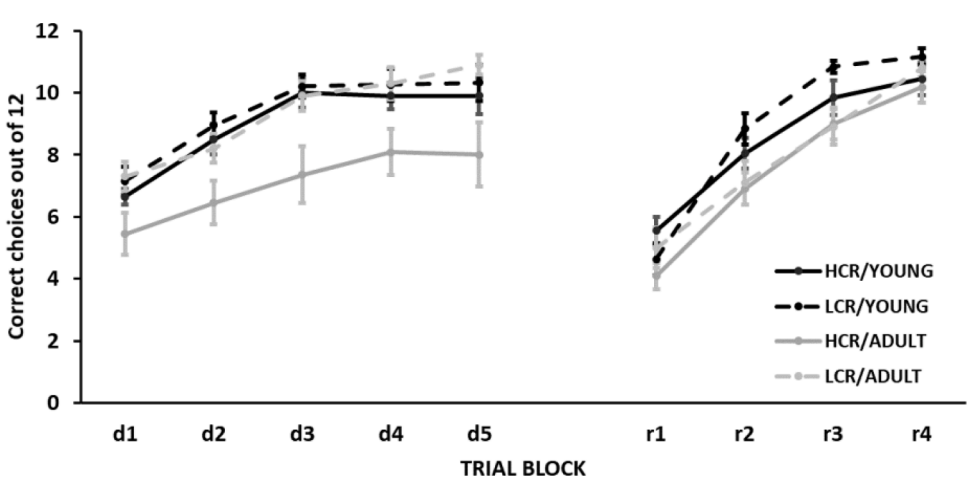

Figure 4. T-maze results. Trial blocks d1 to d5 are discrimination training and trial blocks r1 to r4 refer to rule reversal training. All values are means (+/- SEM).

levels of blood lipids when young [34] indicating the presence of characteristic MetS risk factors. Previously we have shown that adult HCR rats outperform the LCR rats in tasks requiring flexible cognition (rule change) [29]. The present study suggests that there are also differences at lower level of cognition, driven by subcortical, rather than cortical structures.

The clearest difference between the HCR and LCR rats in the open field was in frequency of rearing behavior. In young animals, there were no differences but as a function of age, rearing behavior remained on the same level in HCR but dropped quite a lot in LCR rats. In general, rearing behavior is an indication of information gathering [35]. This, together with the difference in locomotor activity in general suggests that the HCR are more active in exploring their surroundings, or in human terms, more curious. One could speculate that, as the LCR rats do not attend their surroundings as vigorously, they would be poorer learners in spatial learning tasks. The "curiosity pattern" remains even after fasting. When food is served in a novel environment, the HCR tend to explore the surroundings longer before starting to eat [36].

To our knowledge, startle reactivity has not been explored in the HCR and LCR animals before this study. In young animals, there was a general difference in the level of startle response amplitudes, but the habituation itself occurred virtually similarly in both groups. In adult animals, habituation was steeper in the HCR rats compared to the LCR rats. As such, this result would suggest better adjustment in the adult HCR rats as they were better able to ignore the initially adverse but harmless stimulus. Lack of difference in young animals suggests that this adjustment ability deteriorates with age because of the detrimental genome in the LCR rats. In a study by Cooper et al. [37] the LCR had lower threshold to sense mechanical stimuli which can be taken as evidence for higher sensory threshold altogether in the HCR. It should be noted that the HCR rats also have higher pain threshold [38]. However, as the LCR and HCR rats deviate from each other in weight and muscle tone, one should be careful when interpreting results obtained with measures that are based on accelerometer data.

Prepulse inhibition is a phenomenon that reflects sensory gating, i.e. the efficacy of automatic sensory information processing. Deficits of the phenomenon are thought to reflect inability to filter out irrelevant information, most notably so in schizophrenia [39]. The larger the PPI effect (i.e. the drop in the startle amplitude when preceded with a prepulse tone), the better the auditory system can be said to "protect" its own information processing. On the other hand, the faster the PPI effect wears off, the faster the auditory system can be said to have finalized the processing of the prepulse stimulus. In the present study, the LCR and HCR rats differed in the PPI effect from each other, already when young. Even though there was no difference in the magnitude of the PPI effect itself (the startle response was equally and significantly diminished in both lines with a 50-ms lead interval), the effect was longer lasting in the LCR rats. That is, it took a longer time to process the prepulse stimulus in the LCR than HCR rats. In adult animals the difference between LCR and HCR rats was still present but not as clear anymore, as the PPI-effect sustained in both groups beyond the 50 ms lead interval. In sum, we did not find actual deficits in sensory gating in LCR rats, but it seems that the information processing is generally faster especially in the young HCR rats. In the absence of an unselected control group, it is difficult to assess whether the HCR rats are abnormally fast or the LCR rats abnormally slow, but previous studies in other rat strains suggest that the PPI effect is maximal at around 50 ms lead intervals and prevails usually to about 200 ms [40]. There are two factors known to affect the PPI effect: the intensity of pre-pulses and lead interval. Varying the former is a more common practice, but in this case, we chose to test the differences in duration of the putative PPI effect between the rat lines. In future studies, it is perhaps useful to incorporate both factors in the same experiment.

Regarding the T-maze, we did not find very consistent results. Overall, the group that showed the least amount of learning would be the adult HCRs. However, we think it would be a mistake to take the result at face value, because there seems to be a rat line difference in how much the animals are distracted by complex surroundings and/or in what motivates their behavior. First, we observed that rather than pursuing for the food reward, the HCR were paying attention to the experimenter and resisted to move in the maze. In line with this notion, the LCR have been shown to prefer approaching food over investigating the environment when given the choice, which is in contrast with the HCR [36]. Second, in the absence of a human, as is the case in the open field, the HCR were more active and, especially engaged in rearing behavior more readily than LCR. Thus, it might be that the presence or absence of the human experimenter is a factor that plays a role in modifying behavior, especially in the HCR animal. Most publications dealing with behavioral differences between these lines do not explicitly say whether a human was present when the task was applied. This could be one reason for mixed results in learning experiments. As opposed to the present T-maze results, in our previous publication [29], there was a clear difference in favor for the HCR in discrimination-reversal learning. Even though the task was also appetitively motivated, the space in which the animals were tested was much more confined (a closed conditioning chamber). Our recurring observation has been that whenever the experimental settings allow for diverse range of behaviors, the HCR animals might be interested in other things than those that the researchers would wish to assess. In fact, we have recently conducted an experiment where the effect of presence of human on open field behavior was evaluated [41]. We found that the HCR rats decreased moving significantly when the human was present, while in the absence of human the HCR were more active than the LCR. We would suggest that whenever one needs to examine solely cognitive functions of these animals, the number of other distractors should be minimized.

In humans, the presence of MetS symptoms has been linked to a decline in cognitive abilities. [1,2]The literature on cognition and MetS is heavily biased towards adult samples as MetS is usually diagnosed not until later adulthood. Individual MetS risk factors in childhood, especially obesity, are, however, strong predictors of MetS in later life [42]. The present results suggest that those individuals that are genetically prone to MetS might be vulnerable to cognitive challenges already at young age. However, because the link between MetS and cognition in human patients is usually studied using neuropsychological tests or academic success, the direct comparison between the present results and human studies is not possible. In future, it would be of interest to use reflex based testing (such as PPI, eyeblink conditioning, etc.) in human participants with MetS to see whether the effect has some translational value. Especially of interest would be to see whether younger individuals with elevated genetic risk for MetS would show deficits in these cognitive measures.

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