AEROBIC EXERCISE INCREASES HIPPOCAMPAL NEUROGENESIS MORE COMPARED TO RESISTANCE EXERCISE IN RATS SELECTIVELY BRED FOR HIGH/LOW RESPONSE TO TRAINING

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Physical activity is one of the most influential stimulators of adult hippocampal neurogenesis (AHN). Studies have demonstrated that running increases hippocampal neurogenesis and enhances cognitions. Besides aerobic exercise, resistance exercise has been shown to improve cognition but its effect on neurogenesis remains unknown. In addition, individuals vary in their inherent fitness and in their capacity to increase fitness following exercise, but it is not known how these inherent and acquired components of exercise capacity affect hippocampal neurogenesis. The aim of the present study was to examine whether resistance exercise stimulates neurogenesis to an extent comparable to aerobic exercise and whether genetic responsiveness to training affects the rate of neurogenesis. Rats selectively bred for high/low response to training (N=25) were divided into two training groups: aerobic (AER) and resistance (RES). The AER group was exposed to 6 weeks of treadmill running while the RES group was subjected to 6 weeks of resistance training on a vertical ladder. Our results showed that aerobic training increased hippocampal neurogenesis more compared to resistance training. Against our initial hypothesis, there was no difference in the rate of neurogenesis between the strains. Furthermore, we did not find a correlation between training performance (speed/strength) and neurogenesis. Although aerobic exercise is more effective increasing neurogenesis, resistance exercise may induce other brain changes important for cognitive functioning. More research is needed for understanding how different forms of exercise affect the brain and cognition and how genetic components of exercise capacity are involved.

Keywords: hippocampal neurogenesis, aerobic exercise, resistance exercise, HRT/LRT animal model, acquired exercise capacity

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Fyysinen aktiivisuus on yksi keskeisimmistä aikuisiän hippokampuksen neurogeneesiä lisäävistä tekijöistä. Tutkimukset ovat osoittaneet, että juoksu lisää hippokampuksen neurogeneesiä ja parantaa kognitioita. Aerobisen liikunnan lisäksi voimaharjoittelun on todettu edistävän kognitioita, mutta sen vaikutuksista neurogeneesiin ei juurikaan tiedetä. Ihmiset eroavat synnynnäisessä fyysisessä kunnossaan ja siinä, miten hyvin he pystyvät parantamaan kuntoaan harjoittelun seurauksena. Ei kuitenkaan tiedetä, miten nämä harjoittelukapasiteetin synnynnäiset ja hankitut osatekijät vaikuttavat hippokampuksen neurogeneesiin. Tämän tutkimuksen tarkoituksena oli tutkia, vaikuttaako voimaharjoittelu neurogeneesiin samalla tavoin kuin aerobinen harjoittelu ja vaikuttaako geneettinen harjoitteluun reagoivuus neurogeneesin määrään. Korkean ja matalan harjoitteluvasteen mukaan selektiivisesti jalostetut rotat (N=25) jaettiin kahteen harjoitteluryhmään: aerobiseen (AER) ja voimaharjoitteluun (RES). AER-ryhmä suoritti kuuden viikon juoksuharjoittelun juoksumatolla, kun taas RES-ryhmä teki kuuden viikon voimaharjoittelun vertikaalisilla tikapuilla. Tuloksemme osoittivat, että aerobinen harjoittelu lisäsi hippokampuksen neurogeneesiä enemmän kuin voimaharjoittelu. Vastoin alkuperäistä hypoteesia, neurogeneesin määrässä ei ollut eroa kantojen välillä. Emme myöskään löytäneet korrelaatiota harjoittelusuoriutumisen (nopeus/voima) ja neurogeneesin välillä. Huolimatta siitä, että aerobinen harjoittelu on voimaharjoittelua tehokkaampi lisäämään neurogeneesiä, voimaharjoittelu saattaa aiheuttaa aivoissa muita muutoksia, jotka ovat keskeisiä kognitiivisen toiminnan kannalta. Lisätutkimusta tarvitaan ymmärtääksemme, miten eri harjoittelumuodot vaikuttavat aivoihin ja kognitioon ja miten harjoittelukapasiteetin geneettiset osatekijät liittyvät niihin.

Avainsanat: hippokampuksen neurogeneesi, aerobinen harjoittelu, voimaharjoittelu, HRT/LRT -eläinmalli, hankittu harjoittelukapasiteetti

CONTENTS

1 INTRODUCTION

The hippocampus has an integrative role in central nervous system and it is most well-known for its important role in memory and learning (Sørensen, 1985; Squire, 1992). The subgranular zone of the dentate gyrus within the hippocampus is one of the two places where new neurons are produced postnatally in the mammalian brain (Ming & Song, 2005). The process of adult hippocampal neurogenesis (AHN) is influenced by a variety of internal and external factors (Kempermann, 2011).

Physical activity is one of the most effective external factors increasing neurogenesis (van Praag, 2009). Along with neurogenesis, physical activity enhances cognition (for reviews, see Churchill et al., 2002; Sibley & Etnier, 2003). Thus, AHN might mediate the effects of exercise on cognition. Exercise-induced neurogenesis has almost exclusively been studied with aerobic exercise paradigms and little is known about the effects of resistance exercise on the proliferation of new neurons.

There is wide variation between individuals in their inherent aerobic fitness and in their ability to improve fitness following exercise (Bouchard et al., 2012; Timmons et al., 2010). However, relatively little is known whether these inherent or acquired components of exercise capacity affect brain plasticity, such as exercise-induced neurogenesis. It might be that high exercise capacity is associated with beneficial outcomes in the brain.

The aim of the present study is to examine whether resistance exercise stimulates neurogenesis to an extent comparable to aerobic exercise and whether genetic responsiveness to training affects the rate of neurogenesis.

1.1 Adult hippocampal neurogenesis

Adult neurogenesis, a dynamic process of generating functional neurons from progenitor cells (Ming & Song, 2011), has been a topic of controversy. Traditionally, neurogenesis was thought to occur only during embryonic and perinatal phase in the mammalian brain (Ramón y Cajal, 1913). Nowadays it is generally accepted that new neurons are generated

postnatally throughout life in many species including rodents (Altman & Das, 1965; Kaplan & Hinds 1977), birds (Goldman & Nottebohm, 1983), humans (Erikson et al., 1998) and other primates (Gould, Reeves, Graziano, & Gross., 1999).

The subgranular zone of the dentate gyrus within the hippocampus is one of the two regions where neurogenesis occurs in the adult brain (Ming & Song, 2005). The other neurogenic area in the mammalian brain is the subventricular zone of the lateral ventricles. The process of hippocampal neurogenesis starts with proliferation of neural precursor cells in the subgranular zone (Deng, Aimone, & Gage, 2010). Neural precursors become granule cells and migrate into the border of the granule cell layer and hilus. Even though after a few weeks newborn neurons have similar physiological characteristics as mature neurons with dendrites and axons, they still possess stronger synaptic plasticity than mature neurons implying lower threshold for long-term potentiation (LTP) and higher LTP-amplitude. LTP is a prolonged enhancement in signal transmission between two neurons and it is considered to be a synaptic mechanism of learning and memory (Bliss & Collingridge, 1993; Miyamoto, 2006). It has been suggested that these properties of newborn neurons might offer some unique contributions to learning and memory.

The process of AHN (proliferation, survival, migration and differentiation) is regulated by numerous factors from behavioral to molecular level (Kempermann, 2011). Where embryonic neurogenesis is mainly determined by genes, AHN is more activity-dependent and more sensitive to external stimuli even though the molecular mechanisms are fairly similar in both forms. Accumulating evidence has shown that environmental enrichment (Kempermann, Kuhn, & Gage, 1997), physical activity (van Praag, Christie, Sejnowski, & Gage 1999a; van Praag, Kempermann, & Gage, 1999b) and some growth factors such as BDNF, VEGF and IGF-1 (Lee & Son, 2009; LLorens-Martín, Torres-Alemán, & Trejo, 2009) have an up-regulating effect on neurogenesis whereas aging (Kuhn, Dickinson-Anson, & Gage, 1996), stress (Mirescu & Gould, 2006) and sleep deprivation (Guzman-Marin et al., 2005) downregulate the production of new cells. External factors can affect both the proliferation and the survival of new neurons (for a review, see Curlik 2nd & Shors, 2013). For example, aerobic exercise increases the number of proliferating neurons (van Praag et al., 1999b) whereas environmental enrichment (Kempermann et al., 1997) and learning (Shors, Anderson, Curlik 2nd, & Nokia, 2012) increase the number of surviving neurons.

Since hippocampus has a major role in various functions, such as learning, memory and emotion regulation, scientists have become interested in whether AHN is, at least in part, the mechanism underlying these processes. Still, the functional significance of AHN is elusive. Many studies have investigated the functional importance of AHN in learning and memory (for a review, see Deng et al., 2010; Leuner, Gould, & Shors, 2006; Vivar & van Praag, 2013). Shors et al. (2001) were among the first who studied the function of new neurons in learning indicating that newborn neurons play a role in hippocampus-dependent learning. New neurons are also associated with other hippocampus-dependent functions such as spatial learning (Kempermann et al., 1997), pattern separation (Clelland et al., 2009; Creer, Romberg, Saksida, van Praag, & Bussey, 2010) and temporary information storing (Gould, Tanapat, Hastings, & Shors, 1999). However, the relationship between AHN and learning is complex and more research is needed. Environmental factors that enhance neurogenesis also lead to other structural and functional changes in the brain (Zhao, Deng, & Gage, 2008). These changes such as increased neurotrophin levels may contribute to enhanced cognition along with AHN. It remains undetermined whether AHN is the major mechanism underlying improved cognitive functions.

1.2 Exercise, neurogenesis and cognition

Accumulating evidence has shown that physical activity enhances cognition (Churchill et al., 2002; Sibley & Etnier, 2003) and delays age-related loss of cognitive functions (Kramer et al., 1999). Usually, the effects of exercise on cognition have been studied using aerobic exercise programs. Aerobic exercise comprises endurance programs such as running, cycling or swimming (World Health Organization, 2010) and induces metabolic, respiratory and cardiovascular changes in the body (Thomas, Dennis, Bandettini, & Johansen-Berg, 2012). Many studies have demonstrated that aerobic exercise which enhances cardiovascular fitness also improves cognitive functioning in humans (Colcombe & Kramer, 2003; Colcombe et al., 2004; Kramer et al., 1999; Pereira et al., 2007; Stroth et al., 2009). Respectively, animal studies have shown that aerobic exercise has positive effects on cognitive functions, such as learning (van Praag, Shubert, Zhao, & Gage, 2005), spatial memory (for a review, see Voss, Vivar, Kramer, & van Praag, 2013), pattern separation (Creer, et al. 2010), contextual fear conditioning (for a review, see van Praag, 2009) and novel object recognition (for a review, see Voss, et al., 2013). Consequently, it

has been suggested that to achieve an enhancement in cognition, exercise must improve cardiovascular fitness and maximum volume of oxygen consumption (VO_2max).

Besides its beneficial effects on cognition, exercise is one of the most influential stimulators of AHN (van Praag, 2009). Van Praag and colleagues (1999b) were the first to find that running increases neurogenesis in the mouse dentate gyrus. Increased neurogenesis after aerobic training was observed together with improved performance in cognitive tasks (van Praag et al., 1999a). Since then the relationship between aerobic exercise and hippocampal neurogenesis has been established in many animal studies, both with voluntary wheel running (Kobilo et al., 2011; Leasure & Jones, 2008) and forced treadmill training (Kim et al., 2002; Leasure & Jones, 2008; Uda, Ishido, Kami, & Masuhara, 2006). Some studies have demonstrated that the level of neurogenesis is correlated with running distance (Allen et al., 2001; Rhodes et al., 2003) while other studies have not found such correlations (Lee et al., 2013; van Praag et al., 1999b). The enhancing effect of aerobic exercise on neurogenesis is maintained throughout life in rodents (van Praag et al., 2005; Wu et al., 2008). These findings suggest that AHN might be the link between exercise and improved cognitive function.

Even though aerobic exercise is known to enhance neurogenesis, the effect of resistance exercise is unclear. Studies have shown that also non-cardiovascular exercise programs such as resistance and coordination exercise might have beneficial effects on cognitive functioning (for a review, see Hötting & Röder, 2013). Instead of cardiovascular changes, the main objective of resistance exercise is to increase strength, size of skeletal muscles and anaerobic endurance. The beneficial effects of resistance exercise on cognitive functioning has been demonstrated both in human (Cassilhas et al., 2007; Liu-Ambrose et al., 2010; Liu-Ambrose, Nagamatsu, Voss, Khan, & Handy, 2012; Özkaya et al., 2005) and animal (Cassilhas et al., 2012b; Suijo et al., 2012) studies. However, it is not known whether these improvements in cognition are partly due to increased neurogenesis. To our knowledge, Lee and colleagues' (2013) study is the only one concerning resistance exercise and neurogenesis. They found that progressive resistance wheel running, with shorter distances but higher work levels, increased hippocampal neurogenesis comparable to load-free wheel running.

Physical activity provokes several molecular mechanisms which may contribute to cognition via neurogenesis or independently. These molecular mechanisms include growth factors, neurotransmitters and hormones (Hötting & Röder, 2013; Lista & Sorrentino, 2010). Some growth factors like brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF) have been intensively studied regarding neurogenesis and cognition. All of these are up-regulated by exercise and related to enhanced neurogenesis (Ding, Vaynman, Akhavan, Ying, & Gomez-Pinilla, 2006; Bekinschtein, Oomen, Saksida, & Bussey, 2011). In addition, it has been suggested that BDNF and IGF-1 mediate the effects of exercise on cognition. Thus, it might be that these molecular processes enhance both neurogenesis and cognition but the causal relations are unclear. Regarding different types of exercise it has been suggested that aerobic and resistance exercise may both affect cognition but via distinct molecular mechanisms (Cassilhas et al., 2012a). Studies on elderly have shown that resistance training increased peripheral IGF-1 levels but not peripheral BDNF levels (Cassilhas, Antunes, Tufik, & de Mello, 2010; Cassilhas et al., 2007; Correia et al., 2010). Consistent with human studies, animal studies have demonstrated that hippocampal IGF-1 levels are up-regulated by aerobic and resistance exercise but the hippocampal BDNF levels are up-regulated only by aerobic exercise (Cassilhas et al., 2012a). Furthermore, physical activity evokes also other supramolecular mechanisms like angiogenesis and synaptogenesis along with neurogenesis (for a review, see Hötting & Röder, 2013). These all may contribute to each other and cognition. More research is needed for further comprehension of the causal relations.

1.3 Genetic components of exercise capacity

There is wide variation in response to exercise, some individuals showing a great improvement while others show no or only a slight gain (Bouchard et al., 2012; Timmons et al., 2010). Exercise capacity is determined by a complex interaction between genes and environmental factors (Koch & Britton, 2001). The genetics that contribute to exercise capacity consists of an intrinsic (inherent) and extrinsic (acquired) component (Koch, Pollot, & Britton, 2013; Troxell, Britton, & Koch, 2003). Intrinsic genes determine the variation of exercise capacity in the untrained state whereas extrinsic genes determine the adaptive responses to all activity above the sedentary state.

In order to investigate the genetic components of the variation in exercise capacity, selectively bred animal models of intrinsic and adaptational response have been developed. First, Koch and Britton (2001) developed an animal model of intrinsic exercise capacity by divergent selection on the inborn capacity for treadmill running in rats. After 6 generations of selection high capacity runners outperformed low capacity runners in treadmill running in untrained condition. Later, Troxell et al. (2003) developed an animal model of extrinsic exercise capacity by breeding rats according to their adaptive responses to exercise. High response trainers (HRT) and low response trainers (LRT) did not differ in their intrinsic exercise capacity after 15 generations of selection, i.e. there was no significant difference in running distance between the groups before treadmill training (Koch et al., 2013). The difference emerged after treadmill training period indicating different extrinsic exercise capacity.

The intrinsic exercise capacity model has been used to test the aerobic hypothesis, which assumes that the ability to utilize oxygen forms a continuum for health and disease (Koch, Britton, & Wisløff, 2012). Hence, high capacity to utilize oxygen would lead to beneficial health effects and, conversely, low capacity to utilize oxygen would induce multiple health deficits. Studies have demonstrated that low aerobic exercise capacity is associated with many disease risks such as metabolic syndrome and higher mortality whereas improved aerobic fitness is linked to reduced morbidity and mortality levels (Blair et al., 1989).

Even though it is generally accepted that physical activity has beneficial effects on central nervous system, relatively little is known how intrinsic and extrinsic component of exercise capacity affect the brain and cognition. In addition to several disease risks, it might be that low exercise capacity is a risk factor for cognitive deficits. Wikgren and colleagues (2012) demonstrated that rats with high intrinsic exercise capacity outperformed rats with low intrinsic exercise capacity in a task requiring flexible cognition. Study indicates that genetic factors of exercise capacity may have an influence on cognitive functions. Thus, it is possible that differences in cognitive performance are also seen in the brain level, such as differences in the rate of neurogenesis.

1.4 Research questions

In this study we investigate whether resistance exercise stimulates hippocampal neurogenesis to an extent comparable to aerobic exercise. We apply the HRT/LRT animal model to investigate whether acquired exercise capacity affects the rate of neurogenesis. Our hypothesis is that HRT rats are superior in both training forms and, consequently, have more newborn neurons than LRT rats. In addition, we examine whether running speed or strength is correlated with the number of new neurons. Altogether, the aim of this study is to answer the following questions: 1) does a 6- week progressive resistance training regime affect AHN similar to a 6-week progressive aerobic training regime, 2) does the strain, either HRT or LRT, affect the number of new neurons and 3) is there a correlation between training performance (speed/strength) and the number of newborn cells.

2 METHODS

2.1 Animals

The development of the rat model used in the present study has been described in detail earlier (Koch et al., 2013). In brief, selective breeding for extrinsic aerobic capacity was started with a founder population of a widely heterogeneous N: NIH rat stock. In each generation the maximal running distance was tested before and after aerobic treadmill training period. Response to training was calculated as the change in exercise capacity. A within-family selection and rotational breeding paradigm between 10 families was practiced to form contrasting lines of high response trainers (HRT) and low response trainers (LRT).

In the present study we used rats from generation 18 of selection (University of Michigan, Koch & Britton lab). The animals were about nine months old when starting phenotyping protocol. Rats who behaved according to their inherited genotype in the phenotyping were chosen to the next phase of the experiment. These 13 HRT and 12 LRT male rats were used as subjects and exposed either to aerobic (AER) or resistance training (RES) so that they formed four experimental groups: HRT-AER, LRT-AER, HRT-RES and LRT-RES. The rats were individually housed in standard cages approved by the European Union (30 x

18 cm) and kept in 12h - 12h light-dark cycle (lights on 8am - 8pm) with a target room temperature of 22° and humidity of 50%. The rats had ad libitum access to food pellets and water. All experiments were performed in accordance with protocols approved by the European Parliament and the Council of the European Union (directive 2010/63/EU).

2.2 Experimental protocol

The phenotyping and the aerobic training were done on custom-made treadmills (Department of Biology of Physical Activity, University of Jyväskylä) where running space for each rat was 11 x 70 cm. At the end of a lane there was an electrical grid (11 x 11 cm) where the electrical currency was adjustable (0.2 - 2 mA). The vertical ladder used in resistance training was 90 cm high and 15 cm wide (2 cm grid, 85° incline). At the top of the ladder there was a housing chamber (30 x 15 x 11 cm) where rats were able to rest between the trials.

2.2.1 Phenotyping

The protocol included four phases 1) familiarization to the treadmill running, 2) maximal running capacity test before training phase 3) 8 week aerobic training phase and 4) maximal running capacity test after training phase. This phenotyping protocol was a modification from Troxell and colleagues' (2003) protocol.

The first part of the phenotyping consisted of one week familiarization to treadmill. All the rats were habituated to treadmill running, at least on three days for 10 - 20 minutes with max speed which was 10 m/min at the end of the familiarization. If a rat failed to run and slid on an electrical grid at the back of the treadmill, it was gently moved back to the belt.
After the familiarization the rats had at least one day of rest before they performed maximal running capacity tests on three days (Mon, Wed, Fri). Each trial was conducted with a 15° inclination starting with a speed of 10 m/min without a warm-up. The running speed was increased 1 m/min (1.67 cm/s) every 2 minute until the rat was exhausted. The rat was considered exhausted when it remained on the electrical grid for over 5 seconds and refused to run despite of the electrical shock (0.8 -1.0 mA).

The 8 week aerobic training phase was started 2 - 4 days after the maximal tests. On the first day the rats ran at a speed of 10 m/min for 20 min. If more than 90% of the rats

completed the training session, the speed was increased slightly next day. If less than 90% of the rats completed the training, the speed was kept the same or slightly decreased. This procedure was done throughout the 8 week period three times a week. The second maximal running capacity test took place after the training phase. The procedure was the same as in the first maximal test.

2.2.2 Aerobic training

After the phenotyping eight HRT and seven LRT rats who behaved according to their inherited genotype started the 6 week aerobic training protocol. The training was done three times per week with one rest day between the training sessions. The first treadmill training took 25 minutes and the duration of the session was increased every week by 1 min. The speed was kept constant varying from 14 - 22 m/min and with an inclination of 15° uphill. The speed was adjusted for groups so that HRT rats started with a speed of 17 m/min and LRT rats 14 m/min. The velocity was increased 1 m/min every week so that at the end of the training HRT rats run at a speed of 22 m/min and LRT rats run with a speed of 19 m/min. The training protocol was the same for all animals within one group.

The maximal running capacity tests took place every 6th training session. The maximal test replaced the training session from that day. The maximal test was started with a 5-minute warm-up at a speed of 6 - 9 m/min. After the warm-up the maximal test started with a speed of 10 m/min. Then the speed was increased every two minutes with 2.4 m/min (4 cm/s) until exhaustion. No electrical shocks were used in any part of the aerobic training phase.

2.2.3 Resistance training

After phenotyping five HRT and five LRT rats started a resistance training protocol which was a modification from Hornberger and Farrar's (2004) study. The rats were familiarized to a vertical ladder on three days during the first week. On the first day the rats climbed without an extra load and on the next two days with a load of max 50% of a rat's body weight. The load pouch was fixed in the proximal part of the tail with a double-sided tape.
After the familiarization the rats began the 6 week progressive resistance training period. The training session took place three times a week (Mon, Wed, Fri). The first load was 75% of the body weight of a rat. When the rat successfully accomplished to climb with this

load it was increased by 38,9 g. The previous load was increased by this load until the rat could not reach the top of the ladder. The load rat successfully carried to the top of the ladder was considered as a maximal carrying capacity from that session. Subsequent training sessions consisted of 9 trials: 50%, 75% and 90% from previous maximal load. Then the load was increased by 38,9g until the next maximal load was reached. Three trials were aimed to be done with this new maximal load. Between the climbing trials the rats were able to rest 90 sec on the housing chamber located at the top of the ladder. The rats were not punished or rewarded to motivate them to climb in any part of the resistance training phase.

2.3 Tissue preparation

After the experiment rats were anesthetized with carbon dioxide and killed by cardiac puncture. Necropsy was performed immediately and the brain was removed and cut into three pieces (hindbrain, right and left hemisphere). All pieces were immersed in 4% paraformaldehyde for 48 hours and then conserved in 0.1 M phosphate-buffered saline. The right or the left hemisphere was randomly selected and cut coronally with a vibratome (Leica VT 1000s) into 40 µm slices. Every 12th slice was selected for immunohistochemistry to form a representative sample of 9 slices of the whole hippocampus. The slices were stored at -20°C in cryoprotectant solution.

2.4 Immunohistochemistry

Doublecortin (DCX) was used as a marker of newborn neurons. Cryoprotectant was washed away with phosphate buffered saline (0.1 M) three times for 15 minutes. The samples were heated 30 minutes in citrate solution (pH 6) and then chilled in PB-solution. The samples were kept 30 minutes in 1% H_2O_2 phosphate buffered saline and washed in tris buffered saline with Triton X-100 (TBS-T, pH 7.6) three times for 5 minutes. The antibodies used were Doublecortin antibody sc-0866 (Santa Cruz Biotechnology) 1:250 as primary antibody, Biotinylated Rabbit Anti-Goat IgG Antibody (BA-5000, Vector Labs/Mediq) 1:250 as secondary antibody and Streptavidin-Horseradish Peroxidase Conjugate (RPN1231, GE Healthcare/VWR) 1:1000 as tertiary antibody. After each antibody treatment the samples were washed three times for five minutes in TBS-T. The samples were soaked in diaminobenzidine (DAB, D4293, Sigma) with tris buffer and 30% H_2O_2 and washed in 0.1 M Na phosphate buffer. After that the samples were fixed to

microscope slides using gelatin. Cresyl violet was used to stain neurons. Finally coverslips were glued on top of the samples.

2.5 Cell counting

The number of newborn neurons was counted manually with a microscope (Zeiss Primo Star) through a 40x objective within the granular cell layer and hilus in the dentate gyrus. The whole dentate gyrus cell number was estimated by multiplying the cell number of counted nine slices by 24. This estimation of the whole dentate gyrus cell number was used in the analysis.

2.6 Statistical analysis

All statistical analyses were performed with SPSS 20.0. Normality was examined by using Shapiro-Wilks W-test. If variables did not meet the normality criteria, a non-parametric alternative was used. Statistical differences were considered significant when $p < .05$.

The differences between HRT and LRT rats in the phenotyping and aerobic training were tested by using an independent samples t-test. A repeated measures ANOVA was done to examine the interaction between strain and measurement time on maximal strength. To further elucidate the main effect of strain, an independent samples t-test was done separately to all three measurement times. Pearson's correlation coefficient or Spearman's rank correlation coefficient was used to evaluate the relations between different training performance variables.

The effects of strain and exercise on neurogenesis were examined by a two-way ANOVA. Connections between neurogenesis and training performance (speed/strength) measured after training period were examined by Spearman's rank correlation coefficient.

3 RESULTS

All rats from the AER and RES groups successfully completed the 6 week exercise phase. Descriptives about the training performance are presented in Table 1.

Phenotyping. There was no significant difference between HRT and LRT groups in the maximal running speed before phenotyping ($t(23) = 1.39$, $p = .183$). As suspected, a significant difference in the running speed between the two groups emerged after the phenotyping ($t(23) = 2.41$, $p = .029$). The results indicate that HRT rats benefited more from the phenotyping training than did the LRT rats (Table 1).

Aerobic training. There was no statistically significant difference in the maximal running speed between HRT and LRT groups after aerobic training ($t(13) = 1.37$, $p = .195$). On average, both strains performed similarly in the maximal tests (Table 1).

Resistance training. A repeated measures ANOVA showed a significant interaction between the strain and the measurement time on maximal strength ($F[2, 16] = 15.4$, $p = .000$). In order to investigate the main effect of measurement time a repeated measures ANOVA was conducted separately to both strains. The measurement time had a statistically significant effect on strength in HRT ($F[2, 8] = 420.05$, $p = .000$) and in LRT ($F[2, 8] = 161.56$, $p = .000$) group meaning that the both strains increased strength as the training proceeded. The average maximal strength increased from the first measurement to the second (HRT: $F[1, 4] = 293.84$, $p = .000$, LRT: $F[1, 4] = 109.38$, $p = .000$) and from the second measurement to the third (HRT: $F[1, 4] = 177.54$, $p = .000$, LRT: $F[1, 4] = 87.66$, $p = .001$). These results indicate that the 6 week progressive resistance training protocol we adopted successfully increased strength.

As suspected, an independent samples t-test showed that HRT and LRT groups did not differ in strength in the first measurement ($t(8) = 0.21$, $p = .841$). The difference emerged in the second measurement ($t(8) = 4.06$, $p = .004$). Furthermore, the strains performed differently in the third measurement ($t(8) = 4.06$, $p = .004$). The results show that the strength increased more in HRT group and the difference between the strains grew as training proceeded (Figure 1).

Table 1. Descriptives of performance in aerobic and resistance training.

		N	Min	Max	Mean	SD
Running speed (m/m) before phenotyping*						
	both strains	25	20.00	27.00	23.6	1.8
	HRT	13	21.0	25.0	23.1	1.2
	LRT	12	20.0	27.0	24.1	2.2
Running speed (m/min) after phenotyping*						
	both strains	25	22.0	28.0	26.0	1.5
	HRT	13	25.0	28.0	26.7	0.9
	LRT	12	22.0	28.0	25.3	1.8
Running speed (m/min) after aerobic training*						
	both strains	15	26.7	41.1	30.4	3.5
	HRT	8	26.7	41.1	31.5	4.3
	LRT	7	26.7	31.5	29.1	2.0
Load before resistance training (g)						
	both strains	10	340	490	410	58
	HRT	5	360	490	414	65
	LRT	5	340	470	406	57
Load during resistance training (g)						
	both strains	10	700	1000	834	100
	HRT	5	810	1000	912	69
	LRT	5	700	810	756	51
Load after resistance training (g)						
	both strains	10	860	1380	1111	18
	HRT	5	1080	1380	1234	111
	LRT	5	860	1050	988	78

Note. * The results are not fully comparable because after phenotyping the training protocol was slightly modified. In phenotyping training the speed was increased 1 m/min every 2 min. In aerobic training the speed was increased by 2.4 m/min every 2 min.

Correlations of the training performance. Spearman's rank correlation coefficient revealed no significant correlation between maximal running speed after the phenotyping and maximal running speed after the aerobic training ($\rho = 0.39$, $p = .155$). There was neither a correlation between maximal running speed after phenotyping and maximal strength after resistance training ($\rho = 0.22$, $p = .549$). Instead, a significant correlation was found between change (%) in maximal running speed during phenotyping and change (%) in maximal strength during resistance training ($r = 0.74$, $p = .014$) suggesting that rats who increased their speed most during phenotyping increased also their strength most during resistance training.

Hippocampal neurogenesis. A two-way ANOVA revealed no significant interaction between exercise and strain on neurogenesis ($F [1, 21] = 0.11$, $p = .749$) which indicates that the same training form, whether aerobic or resistance, had a similar effect on neurogenesis in both strains. Exercise had a significant main effect on neurogenesis ($F [1, 21] = 5.72$, $p = .026$). On average, AER group had 4341 ± 1418 newborn cells whereas RES group had 3024 ± 1088 cells suggesting that aerobic training enhanced neurogenesis compared to resistance training (Figure 2). Strain had no significant main effect on neurogenesis ($F [1, 21] = 0.23$, $p = .639$).

No statistically significant correlation was found between maximal speed after aerobic training and the number of newborn cells ($\rho = .22$, $p = .426$, $n = 15$). Neither there was a significant correlation between maximal strength after resistance training and neurogenesis ($\rho = .15$, $p = .687$, $n = 10$). The results indicate that the training performance did not affect the number of newborn cells.

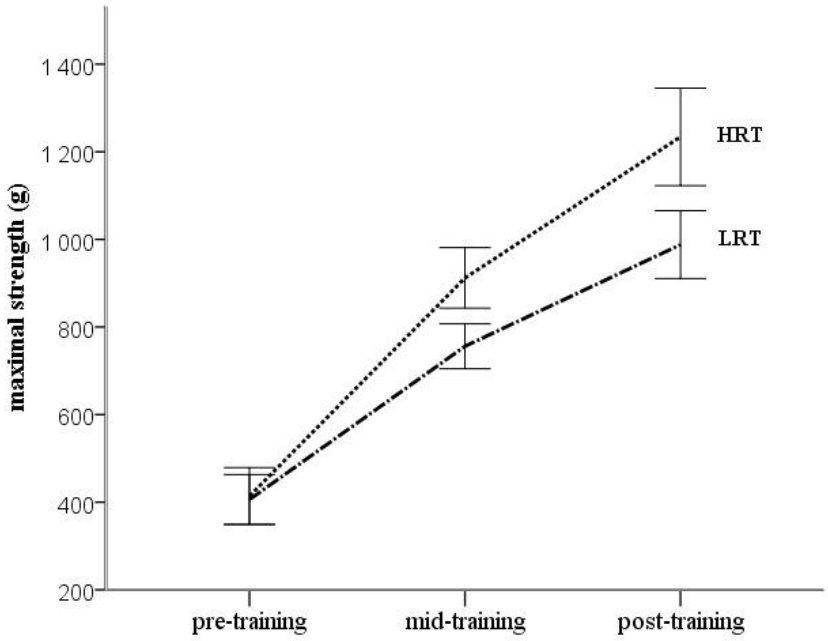


Figure 1. Maximal strength (g) in HRT and LRT groups before, during and after resistance training. All data are presented as the mean +/- SD.

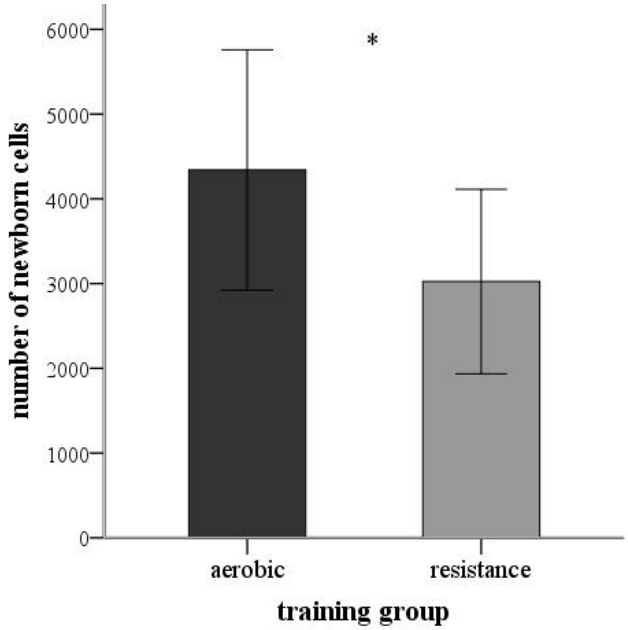
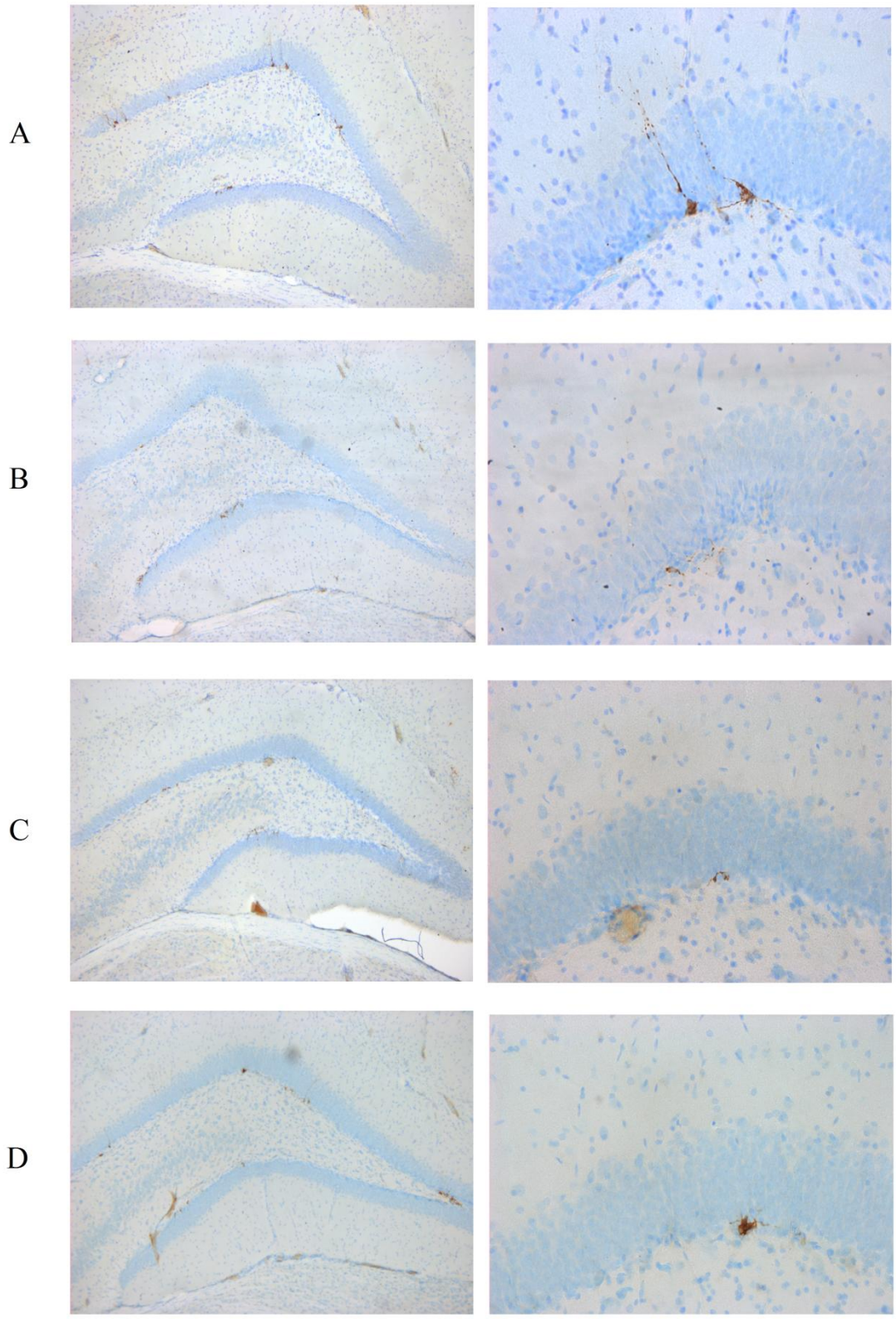


Figure 2. Number of new neurons for aerobic and resistance training groups. (* $p < .05$)



Picture 1. Neurogenesis in the dentate gyrus in different groups. A) AER-HRT, B) AER-LRT, C) RES-HRT, D) RES-LRT

4 DISCUSSION

The aim of this study was to compare the effects of aerobic training and resistance training on AHN and to investigate whether HRT and LRT groups differ in the rate of neurogenesis when exposed to training. We hypothesized that HRT group would produce more new neurons compared to LRT group in both training forms. We also wanted to examine whether there is a correlation between the training performance (speed/strength) and the number of newborn cells.

4.1 Aerobic training enhances neurogenesis more compared to resistance training

Our results showed that the 6 week aerobic training increased neurogenesis in the rat dentate gyrus more compared to the 6 week resistance training. Previous studies have demonstrated that aerobic exercise which induces cardiovascular changes increases AHN (van Praag et al., 1999a,b) but the effects of resistance training remain elusive. To our knowledge, there is one study investigating the effects of resistance training on AHN. Lee and colleagues (2013) found that resistance wheel running (RWR), with shorter distances but higher work levels, increased hippocampal neurogenesis comparable to load-free wheel running. However, RWR did not induce muscle hypertrophy which is a key factor in resistance training. It is possible that RWR caused cardiovascular changes similar to load-free running and thus increased hippocampal neurogenesis. In the present study we wanted to use a training protocol causing physiological adaptations specific to resistance exercise distinct from those of aerobic exercise and to observe its effects on neurogenesis.

A vertical ladder climbing model mimics human resistance training and it has been shown to be effective inducing skeletal muscle hypertrophy (Cassilhas et al., 2012b; Hornberger & Farrar, 2004). In addition, a former study has demonstrated that resistance training on a vertical ladder enhanced cognitive functioning in rats (Cassilhas et al., 2012b). Thus, we concluded that the same training method might induce neurogenesis. Nonetheless, in this study we failed to demonstrate the same amount of neurogenesis after resistance training as after aerobic training.

Studies have shown that the effect of running on neurogenesis peaks at three days and the enhancing effect returns to baseline after 32 days (Kronenberg et al., 2006). However, it is

unclear whether resistance exercise increases neurogenesis and, if so, what is an optimal duration of a training session or the whole training period to increase neurogenesis. It is possible that the time course of the brain changes induced by resistance training differs from those caused by aerobic exercise (for a review, see Voelcker-Rehage & Niemann, 2013). Thus, it might be that our resistance training protocol was not sufficient to induce necessary physiological responses to increase neurogenesis comparable to aerobic exercise. By modifying some features of our protocol, like the duration of a training session or the number of trials, the rate of neurogenesis could have been higher.

Alternatively, it may be that resistance training is not as effective at enhancing neurogenesis as aerobic training is. Studies have shown that both aerobic (Colcombe & Kramer, 2003) and resistance exercise (Cassilhas et al., 2007) enhance cognition. Neurogenesis is thought to mediate the effects of exercise on cognition but the association is demonstrated only in aerobic exercise paradigms. It has been suggested that aerobic exercise and resistance exercise enhance cognition via divergent molecular mechanisms (Cassilhas et al., 2012a). Increased BDNF levels are associated solely with aerobic exercise whereas increased IGF-1 levels have been demonstrated both after aerobic and resistance exercise (Cassilhas et al., 2010; Cassilhas et al., 2012a; Cassilhas et al., 2007; Correia et al., 2010). Molecular mechanisms evoked by aerobic exercise seem to promote neurogenesis more compared to those evoked by resistance exercise. Furthermore, along with neurogenesis, physical activity induces other functional and structural changes in the brain (Hötting & Röder, 2013) so neurogenesis is not necessary for improved cognition (Voss, Nagamatsu, Liu-Ambrose, & Kramer, 2011).

4.2 Extrinsic exercise capacity does not affect neurogenesis

We concluded that HRT rats would improve more than LRT rats in aerobic and resistance training and this would lead to differences in neurogenesis so that the number of newborn cells would be higher among HRT rats. Consistent with a previous study (Koch et al., 2013), HRT and LRT rats run similarly in the maximal test before phenotyping training but HRT rats improved their performance more than LRT rats in the maximal test after phenotyping. This indicates that the strains did not differ in their intrinsic exercise capacity but they had different genetics determining the response to exercise. Interestingly, we failed to demonstrate a difference in the maximal running speed between HRT and LRT

rats after the aerobic training phase. It might be that during the phenotyping HRT rats reached so high level of running capacity that the gains in aerobic training remained marginal. Instead, LRT rats were still able to improve their performance during aerobic training. Accordingly, the difference in the maximal speed between HRT and LRT groups narrowed. The results above are consistent with the result that there was no correlation between the speed after phenotyping and the speed after aerobic training.

As suspected, the strains showed different responsiveness to resistance training. The strength increased more in HRT rats compared to LRT rats and the difference in the maximal load between the strains grew as the training proceeded. The result suggests that the genetic factors influencing adaptive responses to aerobic exercise may be partly the same involved in responses to resistance exercise. Consistent with this is the finding that the rats who increased their speed most during phenotyping increased also their strength most during resistance training. After 8 week phenotyping phase, AER rats continued running whereas RES rats started a new type of training. Thus, as the actual training period begun, AER rats had already adapted to the treadmill training whereas RES rats had no adaptations to resistance training. This might explain why we observed a significant difference between the strains in resistance training but not in aerobic training.

Against our initial hypothesis, HRT and LRT groups did not differ in the amount of newborn cells. We concluded that HRT rats, contrary to LRT rats, would have had high adaptive responses to training and this would have led to high level of exercise-induced neurogenesis. However, the strains did not differ in the aerobic training which might have led to a non-significant difference in neurogenesis. Although the difference emerged in the resistance training performance it might be that resistance exercise does not increase the number of new neurons.

4.3 Correlations

Previous studies have investigated whether there is an association between the amount of running and the number of new cells produced. Some studies have found such correlations (Rhodes et al., 2003) while others have not (Lee et al. 2013; van Praag et al., 1999b). In this study, we used running speed and strength as indicators of training performance. Studies have shown that the variation in running distance varies from one strain to another

(Allen et al. 2001; van Praag et al. 1999b). If inter-individual variation in training performance is small, a possible correlation between neurogenesis and performance is difficult to address in small samples. This might explain why we did not demonstrate a correlation between speed and neurogenesis or between strength and neurogenesis.

Another explanation might be that the relationship between performance and neurogenesis may vanish in rodent models selectively bred for extremities of some feature of exercise. This idea is consistent with the previous study (Rhodes et al. 2003) which demonstrated that mice selectively bred for high levels of voluntary wheel running showed no correlation between running distance and neurogenesis while in outbred control mice the correlation was found. The authors proposed that the lack of correlation in hyperactive mice may be due to their aberrant neurophysiology compared to normal mice. If normal mice could be induced to the same amount of running they might show continuous increase in neurogenesis with the level of exercise without the ceiling effect seen in hyperactive mice. Similarly, in our study it might be that HRT/LRT rats had aberrant neurophysiology so that neurogenesis and training performance were not interconnected as they normally would.

4.4 Evaluation of the research

In this study, we used forced exercise protocols which enable the control of the intensity and the duration of an exercise. Both treadmill and vertical ladder have been used previously and, in addition, treadmill training has been proven to increase neurogenesis (Cassilhas et al., 2012b; Hornberger & Farrar, 2004; Kim et al., 2002; Leasure & Jones, 2008; Uda et al., 2006). Nonetheless, it is worth noting that endurance running and resistance exercise are not the natural way of moving for rodents. Instead, rodents tend to move via brief and rapid runs (Richter, Gass, & Fuss, 2014). Thus, the treadmill and vertical ladder training could represent a stressor for rodents. Moreover, in this study, like in many rodent studies, the experiments were done during the daytime. As nocturnal animals, rats have their active period during the night so experiments done during the day might cause additional stress. Stress is known to downregulate the production of new neurons (Mirescu & Gould, 2006) and this might have had an impact on our results. In later studies it would be useful to evaluate possible stress levels for example by measuring blood corticosterone or adrenal levels.

Adult neurogenesis is typically detected by using thymidine analogs and retroviral labeling. These methods have their limitations, for example retroviral identification requires invasive intracranial injections (Cooper-Kuhn & Kuhn, 2002). In this study we used doublecortin (DCX) which can be used as an alternative indicator of neurogenesis (Brown et al., 2003) without requiring in vivo prelabeling. The expression of DCX is transient and it decreases significantly when new cells begin to express mature neuronal markers. Studies have shown that doublecortin can be detected in progenitor cells and neuroblasts within one month after emerging (Brown et al., 2003). Kronenberg and colleagues (2003) showed that the pro-proliferative effect of exercise peaks at very early stage of training and then after a few weeks it returns to baseline. Therefore, in this study we were not able to investigate the highest peak in exercise-induced neurogenesis because of the total length of the training period (phenotyping 8wk + actual training 6wk). DCX-labeling detects only the cells born 3-4 week before sacrifice. Noteworthy is, that with this labeling method we were able to detect differences in neurogenesis only at the end of the training period. In order to investigate the effect of pro-proliferative period of exercise-induced neurogenesis, the use of BrdU-labeling or shorter training period would be reasonable.

In our study, there are a few limitations that future studies can address. First, in the present study we did not apply a sedentary control group. The inclusion of the sedentary control group would have enabled the investigation of whether resistance training increases neurogenesis compared to sedentary state. Second, the effectiveness of the training could have been evaluated in more detail. For example, the investigation of the skeletal muscle hypertrophy would have given us information whether resistance training increased muscle size. If hypertrophy did not happen, resistance training might have been too low-intensity to cause necessary physiological changes in the periphery to induce neurogenesis. Thirdly, it might be that the phenotyping training affected rats' performance in the actual training. HRT and LRT rats could have performed differently both in aerobic and resistance training without the phenotyping and this might have led to differences in the rate of neurogenesis. In subsequent studies the effect of strain on neurogenesis could be studied without the phenotyping training or shortening the total length of a training period.

Although it is generally accepted that physical activity enhances cognitive functioning, it remains elusive which physiological mechanisms mediate the effects of exercise on

cognition. Neurogenesis may be one of the mechanisms, but not the only one. The situation is even more complicated when different forms of exercise are concerned. In order to understand the complexity of physiological changes involved in improved cognition, more research is needed. Future studies could compare the effects of aerobic training and resistance training on plasticity related molecules and to investigate their effects on neurogenesis and cognition at the same time. Furthermore, animal models should be exploited for further understanding about the genetic components of exercise capacity in health and disease. Even though the results from the animal studies are not directly transferable to humans, animal models help to discover the basic brain changes related to physical exercise. Since hippocampus has a major role in learning, memory and emotional behaviour, the investigation of adult hippocampal neurogenesis may clarify its functional significance in these processes and enable the development of new interventions for learning disabilities, neurodegenerative diseases and affective disorders.

4.5 Conclusions

This study was among the first comparing the effects of aerobic and resistance exercise on hippocampal neurogenesis and assessing the effect of extrinsic exercise capacity on the number of new neurons. The present study showed that aerobic exercise increases neurogenesis more compared to resistance exercise. This supports the idea that physical exercise enhancing cardiovascular fitness increases hippocampal volume and improves cognitive functioning (Erickson et al., 2011). Although aerobic exercise enhances neurogenesis more, resistance exercise may induce other changes important for cognitive functioning. Moreover, this study gave additional information that the genetics determining the responses to aerobic exercise might be partly the same involved in responses to resistance exercise. However, we failed to demonstrate that extrinsic exercise capacity affect the rate of exercise-induced neurogenesis. The results of this study should be confirmed in subsequent studies with larger sample sizes.

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