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Exercise-induced microRNA modulations and their role in cognition

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List of Abbreviations

AGO	Agronaute
ANOVA	Analysis of variance
Anti-miR-409-5p	microRNA-409-5p inhibitor
Anti-miR-501-3p	microRNA-501-3p inhibitor
Anti-miR-100-5p	microRNA-100-5p inhibitor
ApoE	Apolipoprotein E
APP/PS1	Amyloid precursor protein and presenilin 1
BDNF	Brain-derived neurotropic factor
Bp	Base pairs
C57Bl/6	C57Black/6
C9orf72 or C9	Chromosome 9 open reading frame 72
CA1	Cornu Ammonis 1
cDNA	Complementary deoxyribonucleic acid
Cor	Correlation
DGCR8	Drosha and DiGeorge syndrome critical region 8 protein
DGN	Die Deutsche Gesellschaft für Neurologie
DGPPN	Die Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde
DiI	Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate
DIV	Day in vitro
DMEM	Dulbecco's Modified Eagle Medium
DNase	Deoxyribonuclease
DPBS	Dulbecco's phosphate-buffered saline
E17	Embryonic stage 17
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
GC	Guanin-Cytosine
GRN	Progranulin
Hsa	*Homo sapiens*
IGF-1	Insulin-like growth factor 1
LNP	Lipid nanoparticle
LTM	Long-term memory
MAP2	Microtubule-associated protein 2
MAPT	Microtubule associated protein tau
ME	Modular eigengene
MEA	Microelectrode array
microRNA	Micro-ribonucleic acid
Mmu	*Mus musculus*
NMDA	N-methyl-D-aspartate

nt	Nucleotides
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PDL	Poly-D-Lyine
PF4	Platelet factor 4
PSD-95	Postsynaptic density 95
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
STM	Short-term memory
TMT-A	Trail Making Test A
TMT-B	Trail Making Test B
UTR	Untranscribed region
VEGF	Vascular endothelial growth factor
VLMT	Verbal Learning Memory Test
WCST	Wisconsin Card Sorting Test
WHO	World Health Organisation

1 Introduction

Loss of cognitive function, a typical symptom of dementia, has become a great challenge for humankind recently. The number of people suffering from dementia is constantly increasing leading to massive long-term economic and social consequences. Numerous attempts have been made to understand the mechanisms of cognitive decline and to find an efficient approach to improving it. A healthy lifestyle and physical exercise in particular are well known for their positive effects on brain functions. The molecular mechanisms of this effect, however, remain poorly understood. Epigenetics can serve as a key in understanding how lifestyle factors mediate long-term phenotypic alterations. Micro ribonucleic acid (microRNA), one of the epigenetic regulators, is a very promising target for investigating the mechanisms of cognitive improvement and for potential therapy.

1.1 Background

1.1.1 Epidemiology of cognitive impairment

Cognitive impairment is a very common condition in elderly. Around 15 % of people older than 70 are being diagnosed with dementia, a major neurocognitive disorder (Plassman et al. 2007). One person every three seconds - this is the dismal trend we are currently experiencing (Prince et al. 2015). On top of that, it is believed that just as many people suffer from symptoms of mild cognitive impairment which does not meet the criteria of dementia as a diagnosis (Knopman and Petersen 2014).

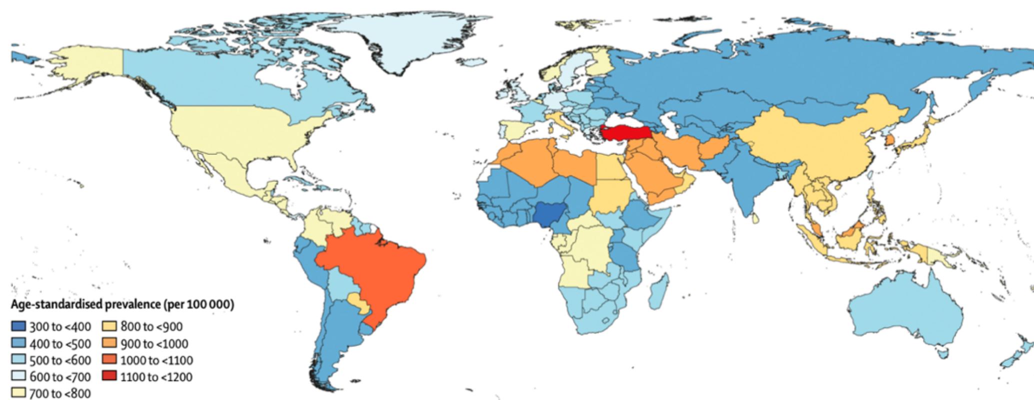

Figure 1: Prevalence of dementias per 100000 population in the world. Figure copied from Nichols et al. (2019). CC BY 4.0

There are currently approximately 50 million patients diagnosed with dementia and this number is expected to increase almost threefold by 2050 (Prince et al. 2015). The estimated prevalence of dementia is 593 per 100000 population in the world (Figure 1) (Nichols et al. 2019). In Germany there are 1.7 million people suffering from dementia (Stock et al. 2018). Dementia and mild cognitive impairment are very costly as these patients have a greater risk of hospitalization (Lin et al. 2017). Dementia as a group of different diseases, including the most common form of it Alzheimer's diseases, is estimated to be the fifth leading cause of death worldwide (Nichols et al. 2019).

1.1.2 Current treatment of cognitive impairment

To date no treatment exists to cure dementia. Several drugs can be considered as an option to slow down the process of cognitive decline such as cholinesterase inhibitors, the noncompetitive N-methyl-D-aspartate (NMDA) antagonist memantine (WHO 2017) and additionally Ginkgo Biloba based on current German S3 guidelines (DGPPN and DGN 2016). The absence of efficient medication and multimorbidity of many geriatric patients has led professional societies to search for good non-drug alternatives, where they pay lots of attention to the effectiveness of a healthy diet and an active lifestyle (von Arnim et al. 2019; WHO 2019).

1.1.3 Effects of physical activity on cognitive impairment

It has long been recognized that physical activity boosts brain function (Pierson and Montoye 1958; van Praag et al. 1999). Considering that current pharmacological options cannot cure but rather modulate the symptoms and may cause adverse effects, exercise has been presented as an effective and safe alternative, promoting ever more and more research of this topic. There has been much debate over the most efficient type of exercise, however, based on the latest meta-analysis, short and intensive session may bring the best outcome for older patients with cognitive impairment (Sanders et al. 2019).

Many authors have observed in imaging studies structural changes associated with physical activity in areas responsible for attention and memory such as the hippocampus, the temporal and frontal lobes (Colcombe et al. 2006; Erickson et al. 2009; Maass et al. 2016). Moreover, induced corticospinal excitability was observed after exercise (Singh et al. 2016). The cerebral blood flow was also shown to be induced, stimulating the process of neurogenesis after physical loading (Pereira et al. 2007). In animal models it has been known for a long time that running induces neurogenesis in germinative zones (van Praag et al. 1999; Fischer 2016).

Many attempts have been made to understand the mechanisms behind these changes. The synthesis of brain-derived neurotropic factor (BDNF) (De la Rosa et al. 2019), insulin-like growth factor 1 (IGF-1) (Maass et al. 2016), vascular endothelial growth factor (VEGF) (Fabel et al. 2003) and platelet factor 4 (PF4) (Leiter et al. 2019) from the periphery may induce the changes in the brain. Neuroinflammation is believed to play role in memory deterioration in dementia (Baune et al. 2008; McAfoose and Baune 2009). Aerobic exercises were also associated with decreased levels of inflammation in the brain and improved memory in animal models (Tapia-Rojas et al. 2016; Do et al. 2018). It is worth noting that all the studies succeeded in explaining the mechanisms of cognitive improvement on a deep molecular level.

1.1.4 MicroRNA

One of the epigenetic regulators is a small non-coding microRNAs whose length typically does not exceed 22 nucleotides (nt). By interacting with many target messenger RNAs they modulate gene expression (Gebert and MacRae 2019).

The microRNA gene is initially transcribed into primary immature microRNA mostly by polymerase II (Winter et al. 2009). Later it undergoes a process of maturation where it consequently becomes the first precursor microRNA and later the mature microRNA. The primary microRNA hairpin is excised in the nucleus by the RNase III Drosha and DiGeorge syndrome critical region 8 protein (DGCR8), also known as Pasha (Lee et al. 2003; Denli et al. 2004). Then, the precursor microRNA hairpin is transported from the nucleus to the cytoplasm via the protein Exportin (Okada et al. 2009). Finally, in cytoplasm, the precursor microRNA is cleaved by the enzyme Dicer and becomes a mature double-stranded microRNA, comprised of one 5p and one 3p strand (Zhang et al. 2004; Nicholson 2014). One so-called functional or guide strand is loaded into Agronaute (AGO), mostly AGO2, and binds to 3p untranscribed region of the target mRNA leading to gene silencing by repression of translation, while the other is being degraded (Matranga et al. 2005; Guo et al. 2010; Jonas and Izaurralde 2015; Gebert and MacRae 2019) (Figure 2). In contrast, microRNA can also increase the translation rates in certain conditions (Vasudevan et al. 2007; Vasudevan 2012).

Interestingly, one microRNA can interact with several messenger RNAs (Vasudevan 2012; Ha and Kim 2014). MicroRNAs are involved in many essential physiological processes such as embryogenesis, cell differentiation and homeostasis (Bernstein et al. 2003; Chong et al.

2010; Pauli et al. 2011). MicroRNA structure as well as their targets are highly conserved in mammals (Bartel 2004; Friedman et al. 2008). This fact provides us with a good presumption that molecular mechanisms that are regulated by the same microRNAs can be safely investigated in a mouse model. Moreover, they are excreted into the blood stream in microparticles or in free-soluble form allowing them to implement their function at great distances from the initial area of synthesis and working in a hormone-like manner (Bayraktar et al. 2017; Huang 2017). Finally, they have been found to be involved in numerous pathological conditions, including neurological diseases (Esteller 2011).

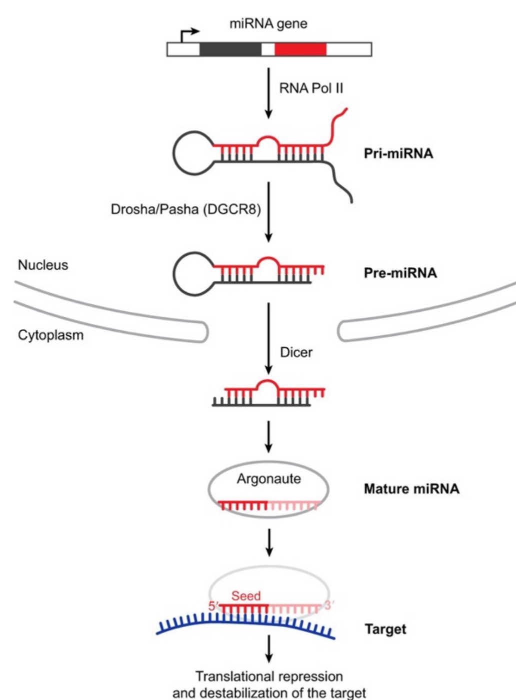

Figure 2: MicroRNA synthesis. Transcription of microRNA by polymerase II into primary microRNA (pri-miRNA) hairpin. The Drosha/Pasha (DGCR8) complex processes it further to precursor microRNA (pre-miRNA) hairpin. After leaving the nucleus via Exportin (not shown) pre-miRNA is processed by the Dicer enzyme and becomes mature double-stranded microRNA. One strand is loaded on Agronaute and binds to the target where it implements its functions. Figure reproduced with permission from The Company of Biologists Ltd., Alberti and Cochella (2017).

1.1.5 MicroRNA and exercise

The first thing that comes to mind when we think about exercising is its effect on muscle growth and improved oxygenation. It has been observed that after aerobic exercise there is an increase in expression in elements of the microRNA synthesis pathway such as Drosha, Exportin and Dicer (Russell et al. 2013). Indeed, microRNAs were found to be involved in such adaptive processes as cardiac muscle growth, skeletal muscle growth and the stimulation

of angiogenesis in human and animal models (Silva et al. 2017). Moreover, some microRNAs are proposed as a biomarker for physical response and adaptation after and during exercises of different modalities as key regulators of Phosphoinositide 3-kinase Protein kinase B mammalian target of rapamycin signaling cascade that in turn regulates muscle growth, cell proliferation and differentiation (Domańska-Senderowska et al. 2019).

Many studies were performed in a mouse model after exercise in order to investigate the microRNA profiles and the effects on the heart. It was shown that microRNA-26b, -150, -27a and -143 are involved in physiological left ventricle hypertrophy induced by wheel running (Martinelli et al. 2014). MicroRNA-222 is involved in myocardial growth and prevents its pathological remodeling after voluntarily wheel running (Liu et al. 2015). MicroRNA-21 and -30b were upregulated in heart tissues and associated with cardiac apoptosis prevention after swimming (Zhao and Ma 2016). MicroRNA-17-3p is associated with myocardial growth and protection against ischemia (Shi et al. 2017). Based on rat studies, microRNA-126 stimulates VEGF pathway and induce angiogenesis in heart (Da Silva et al. 2012).

Alterations in microRNA expression levels were also found in muscle biopsies in the mouse model. MicroRNA-1, -23, -107, -133 and -181 were upregulated and associated with adaptive response on acute endurance training (Safdar et al. 2009). MicroRNA-133b is responsible for exercise capacity and mitochondrial number (Nie et al. 2016). MicroRNA-181b-5p was found in mouse skeletal muscle and is believed to be associated with acute maximal exercise (Shah et al. 2017). Similar results were found in human muscle tissue after exercise, namely microRNA-1, -29b -133a, -133b, 181a. Some of them were described as regulators in muscle regeneration and mitochondrial synthesis (Russell et al. 2013). MicroRNA-1, -133a, -133b, -206 were found in the vastus lateralis muscle after short- and long-term training (Nielsen et al. 2010). MicroRNA-451 was associated with better response on exercise, meaning more efficient muscle growth (Davidsen et al. 2011). Other microRNA, namely -136, -200c, -376a, -377, -499b, -558 were also associated with muscle hypertrophy (Ogasawara et al. 2016).

Many human studies were published on the circulating microRNA profile of different training types. Some of the studies were done purely from a sport medicine perspective searching for a biomarker of systemic response to exercise, some were done with the purpose to correlate with some improved physiological parameters. For our study, the

most relevant are microRNAs that were found to be upregulated in blood after aerobic training (Table 1)

Table 1: Upregulated circulating microRNA after exercises

microRNA	Sample	Reference
-125a, -145, -181b, -193a, -197, -212, -223, -340, -365, -485, -505, -520d, -629, -638, -939, -940, -1225, -1238	Circulating neutrophils	(Radom-Aizik et al. 2010)
-7, -15a, -21, -26b, -132, -140, -181a, -181b, -181c, -338, -363, 939, -940, -1225	Circulating mononuclear cells	(Radom-Aizik et al. 2012)
-let-7f-1, -21, -29c, 223	Circulating mononuclear cells	(Dias et al. 2015)
-7, -29a, -29b, -29c, -30e, -142, -192, -338, -363, -590	Circulating natural killer cells	(Radom-Aizik et al. 2013)
-15a, -29b, -29c, -30e, -140, -324, -338, -362, -532, -660, -1202, -1305	Circulating monocytes	(Radom-Aizik et al. 2014)
-133b, -181a	Skeletal muscle extracellular vesicles	(Guescini et al. 2015)
-92a, -92b	Whole blood	(Taurino et al. 2010)
-1, -486, -494	Whole blood	(Denham and Prestes 2016)
-20a, -21, -146a, -221, -222	Plasma	(Baggish et al. 2011)
-1, -133a, -133b, -181b, -208b, -214	Plasma	(Banzet et al. 2013)
-103, -107, -139, -143, -223, -330, -338	Plasma	(Nielsen et al. 2014)
-1, -133a, -133b, -206, -485, -509, -517a, -518f, -520f, -522, -553, -888	Plasma	(Cui et al. 2016)
-376a	Plasma	(Zhang et al. 2017)
-140, -221, -223	Plasma	(Parr et al. 2016)
-181b, 146a, -127-3p, 197-3p, 148a	Plasma	(Shah et al. 2017)
-210, -222, -21	Plasma	(Bye et al. 2013)

microRNA	Sample	Reference
-133a, -206	Plasma	(Danese et al. 2018)
-149	Serum	(Sawada et al. 2013)
-19a-3p, -19b-3p, -20a, -26b, -143-3p, -195	Serum	(Margolis et al. 2016)
-142-3p, 221-3p, 126-3p, 146a-5p, 27b-3p,	Serum	(Barber et al. 2019)

Mature microRNA is derived from 5 prime end of the precursor duplex if not stated otherwise.

We know now that there are epigenetic changes happening in response to physical activity. Unfortunately, not much is known about their mechanistic function. Some of these microRNAs may at the same time play a role in cognitive stimulation.

1.1.6 MicroRNA and cognition

MicroRNAs play an important role in neuronal growth, differentiation and synaptic plasticity during physiological development (Sempere et al. 2004; Schratt et al. 2006; Dogini et al. 2008; Hu et al. 2015) as well as in neurological diseases (Barbato et al. 2008). Interestingly, some microRNAs, including those differentially expressed after exercise based on separate studies, regulate previously mentioned genes that are involved in exercise response such as BDNF, IGF-1, VEGF and others that are believed to stimulate brain function (Mellios et al. 2008; Fenn et al. 2013; Zhao et al. 2019).

As mentioned above, microRNAs are very stable in liquid biopsies. Looking for a reliable biomarker of cognitive impairment led to establishing microRNAs as promising candidates. Several differentially expressed microRNAs were found (Table 2). Such changes in blood may represent the pathology happening in the brain. Nevertheless, the mechanisms of these changes remain poorly understood. The above-mentioned microRNAs were found to be mainly downregulated in diseases with cognitive decline. It is important to test if the same microRNAs that can serve as a biomarker of cognitive impairment are oppositely changed after physical loading. If so, they can be considered as therapeutic targets.

Table 2: MicroRNA signatures in different conditions associated with cognitive decline

microRNA	Sample	Condition	Expression	Reference
-let-7g, let-7e, -let-103a	Serum, plasma, blood	Alzheimer's disease	Decreased	(Kumar et al. 2013; Leidinger et al. 2013; Satoh et al. 2015)
-let-7g, let-7e, -let-103a, -99b, -19a-3p	Serum exosomes	Aging	Increased	(Rani et al. 2017)
-409-3p	Plasma	Vascular dementia	Decreased	(Prabhakar et al. 2017)
-409-5p	Mouse brain	APP/PS1 mouse model*	Decreased	(Luo et al. 2014; Guo et al. 2019)
-let-7d, -409-5, -9, -143, -146a	Rat brain	Ages rats with isoflurane-induced memory loss	Decreased	(Luo et al. 2015)
-30b, -18a, -103a -142-3p, -151a, -301a	Plasma	Alzheimer's disease, mild cognitive impairment	Decreased	(Nagaraj et al. 2017)
-501-3p, -let-7f, -26b	Serum	Alzheimer's disease	Decreased	(Hara et al. 2017)
-501-3p	Mouse brain	Mouse model for vascular dementia	Increased	(Toyama et al. 2018)
-99b, -100, -501-3p	Mouse brain	APP/PS1 mouse model	Decreased at early stages	(Ye et al. 2015)
-193b	Serum exosomes	APP/PS1 mouse model, Alzheimer's disease, mild cognitive impairment	Decreased	(Liu C-G et al. 2014a)
-384, -200b	Plasma, serum	Alzheimer's disease, mild cognitive impairment	Decreased	(Liu C et al. 2014; Liu C-G et al. 2014b)
-31, -93, -143, -146a	Serum	Alzheimer's disease	Decreased	(Dong et al. 2015)

microRNA	Sample	Condition	Expression	Reference
-210	Serum, cerebrospinal fluid	Alzheimer’s disease	Decreased	(Zhu et al. 2015)
-29a, -29b-1	Brain	Alzheimer’s disease	Decreased	(Hebert et al. 2008)

Mature microRNA is derived from 5 prime end of the precursor duplex if not stated otherwise.

* APP/PS1 is a double transgenic mouse model for Alzheimer’s disease with mutated overexpressed amyloid precursor protein and presenilin 1.

One recent study investigated specifically microRNA deregulation in mouse hippocampus after physical exercise. They performed small RNA sequencing and identified the following microRNAs associated with improved memory retention: microRNA-129-1-3p, microRNA-144-5p and microRNA-708-5p (Fernandes et al. 2018).

So far no one has investigated alterations in circulating microRNA caused by physical exercise and their association with cognition. It is known that exercise boosts cognition even in healthy individuals (Sanders et al. 2019). Modern sequencing technologies allow us to interrogate the entire microRNAome and identify expression differences for each microRNA. We believe that there are certain microRNAs that appear in blood after physical activity that somehow affect gene expression in the brain that in turn improves cognitive function. Some of them might be even oppositely expressed compared to diseases with cognitive decline. If we prove this concept, these microRNAs can help us understand how to therapeutically improve deteriorated cognitive functions. Finding associated microRNA signatures, their modulation in experimental models and understanding the mechanisms of action may reveal therapeutic potential against diseases characterized by cognitive decline.

1.2 Research aims and objectives

The aim of this project is to investigate the role of circulating microRNAs associated with regular physical activity in cognition.

For this purpose, we aimed to analyze microRNAome of whole blood samples from healthy volunteers collected before and after three-month long regular endurance training and then, based on a rigorous analytical approach, to select potential microRNA candidates associated with improved cognition. These observations will be compared *in vitro* and *in vivo* exercise models. Selected microRNAs will be latter manipulated in different experimental models and neuronal morphology and functions will be tested. Compiled panel of microRNAs will be also tested in samples from patients with diagnosed cognitive impairment.

2 Materials and Methods

2.1 Participants

Within the framework of a previously published clinical study 19 (14 men and 5 women) healthy volunteers were recruited between 2010 and 2013 in the Department of Psychiatry and Psychotherapy at the University Medical Center Goettingen for schizophrenia study (Table 3). The inclusion criteria were following: age matched individuals, no medical history of mental disorder and no participation in exercise studies two years prior the mentioned study (Malchow et al. 2015). These subjects were part of the control group in several studies investigating the effect of physical training on cognitive function (Malchow et al. 2015; Malchow et al. 2016; Rauchmann et al. 2019; Roeh et al. 2020). The study was approved by the local ethics committee of the University Medical Center Goettingen and was in accordance with the declaration of Helsinki. All volunteers provided an informed consent prior to the beginning of the study.

Table 3: Participants and their clinical characteristics

Parameter	Mean (SD)
Age, years	39.1 (10.1)
Height, cm	175.7 (9.7)
Weight, kg	81.3 (13.7)
Waist, cm	89.5 (12.7)
Education, years	17.1 (4.1)
Systolic blood pressure, mmHg	132.5 (12.7)
Diastolic blood pressure, mmHg	77.7 (8.8)
Pulse, bpm	73.4 (10.6)
Cigarettes per day, number	5.4 (8.9)

SD: standard deviation

2.2 Endurance training and blood collection

Healthy individual participated in twelve weeks of endurance training. The physical exercise was performed three times a week for 30 min on bicycle ergometer machines (ergobike Premium 8, Daum electronic GmbH, Fuerth, Germany). The intensity of sessions was

defined individually and was gradually increased according to blood lactate concentration, gas exchange, heart rate and exhaustion. A detailed protocol for aerobic training conducted in the mentioned study can be found elsewhere (Malchow et al. 2015; Malchow et al. 2016). Blood was collected at start and end point of the whole experiment. The whole blood was withdrawn from cubital vein and stabilized into PAXgene® Blood RNA tubes (BD, Germany). Tubes were left at room temperature for at least 2 h to secure the total lysis of blood cells. The samples were then transferred to -20 °C for 24 to 72 h and then transferred to -80 °C freezer for final storage and preservation of microRNA expression profile.

2.3 Cognitive testing

Cognitive tests before the beginning of training and after regular aerobic exercise were performed as described before (Malchow et al. 2015). Processing speed and executive function were analyzed by Trail Making Test A (TMT-A) and Trail Making Test B (TMT-B) (Reitan and Wolfson 1985). For measuring cognitive flexibility the Wisconsin Card Sorting Test (WCST) 64 was used (Kongs et al. 2000). In order to test short-term memory (STM) and long-term memory (LTM) Verbal Learning Memory Test (VLMT) was performed (Müller et al. 1997). The difference between the baseline at the beginning and the last results after three-month of training was analyzed in our study.

2.4 Animals

Two months old male and female C57 Black(Bl)/6 (Janvier Labs, France) were used in the current study for exercise experiments. The body weight was approximately 20-25 g. All animals were housed in single type II cages ($350cm^2$ x 14cm) with additional enrichment. Time-pregnant mice with CD-1® background (Janvier Labs, France) were used for primary neuronal culture experiments. In the animal facility, the artificial 12 h light and 12 h dark cycle was maintained. Mice were provided with food and water *ad libitum*. All animal experiments were performed according to the local guidelines and were approved by the local Animal Welfare Office of Goettingen University and the Federation of European Laboratory Animal Science Associations. Prior to the beginning of the experiments a certificate on theoretical qualification pursuant to the ordinance on animal protection in experiments for persons who conduct animal experiments, with species-specific contents on mouse, rat and rabbit from 30.11.2018 was obtained. All animal experiments were performed under approved protocols in accordance to local animal welfare regulation governing experimental animals.

2.5 Mice exercise experiment

C57Bl/6 mice were split into sedentary and exercise groups (n = 10/10). Cages in both groups were identically enriched and contained running wheels. In sedentary group however the wheels were blocked so that running was not possible. The total period of experiment lasted 18 weeks. During that time the exercising group had permanent access to running wheels. The information from the wheels could be constantly monitored in order to later exclude potential non-running animals.

2.6 Collection of brain CA1 subregions

At the end of the 18 weeks of the exercise experiment, C57Bl/6 mice were sacrificed by cervical dislocation. The whole brains were isolated. The dissection of the Cornu Ammonis 1 (CA1) region was performed under a Leica MC170 HD stereo microscope using fine microsurgical instruments. The whole procedure was performed in ice-cold Dulbecco's phosphate-buffered saline (DPBS, PAN-biotech GmbH, Germany) supplemented with Ethylenediaminetetraacetic acid(EDTA)-free protease inhibitor cocktail (Roche Diagnostics GmbH, Germany). Fresh tissues were then snap frozen in liquid nitrogen and stored at -80 °C.

2.7 Mouse primary neuronal cultures

Pregnant CD-1® mice were sacrificed by cervical dislocation. Pups at the embryonic stage 17 (E17) were quickly decapitated. Hippocampi were dissected using a Leica MC170 HD microscope and pulled in a 15 ml tube filled with ice-cold DPBS (PAN-biotech GmbH, Germany). At the end of the dissection, ice-cold DPBS was exchanged with pre-warmed DPBS and 2.5 % trypsin-EDTA (Gibco, USA). In this solution the samples were incubated in a water bath at 37 °C for 15 min. After that the reaction was stopped by adding and mixing several times with warm processing media containing Neurobasal® medium 1x (Gibco, USA), 10 % fetal bovine serum (FBS, Gibco, USA) and 1 % Penicillin-Streptomycin solution (Thermo Fisher Scientific Inc, USA). Next the tissue was mechanically homogenized with the pipet and centrifuged in processing media for 5 min at 300 × g. The cell pellet was resuspended in maintenance media containing Neurobasal® medium 1x (Gibco, USA), 2 % B-27™ supplement (Gibco, USA), 1 % Penicillin-Streptomycin solution (Thermo Fisher Scientific Inc, USA) and 1 % GlutaMAX supplement (Gibco, USA) and filtered with 70 µm Falcon™ cell strainer (Fisher Scientific, USA). Neubauer chamber was used to count the cell

number. Cells were then resuspended in an adjusted volume of maintenance media and seeded with density 130000 cells/well on 24 well plates. For the experiments including image acquisition, sterile glass cover slips with a diameter 12 mm were placed before coating. The plates were prepared in advance by coating with 0.5 mg/L poly-D-Lyine (PDL, Sigma-Aldrich, Germany), incubating at 37 °C for 1 h and following washing with distilled water three times. 30 % of the volume was changed every third day. Cell cultures were treated at day in vitro (DIV) 10 or DIV 7 for 48 h for RNA sequencing and imaging analysis respectively.

2.8 Mouse muscle cell line differentiation

For experiments with murine muscle cell commercially available immortalized myoblast cell line C2C12 was ordered (Sigma-Aldrich, Germany). Cells were first seeded on 25 cm cell culture flasks (Sigma-Aldrich, Germany) and cultured in growth media containing Dulbecco's Modified Eagle Medium (DMEM) without sodium pyruvate, but with high glucose (Gibco, USA), 1 % Penicillin-Streptomycin solution (Thermo Fisher Scientific Inc, USA) and 10 % FBS (Gibco, USA). When cells reached approximately 80 % confluency, they were detached using 0.05 % Trypsin-EDTA (Thermo Fisher Scientific Inc, USA) and were passaged to six-well plates for differentiation experiments. For differentiation of myoblast to myotube, cells were subjected to low-serum media containing 2 % horse serum (Gibco, USA) when they reached 80 % confluency. This media was completely changed every day for five subsequent days. In the evening of the fifth day, the media was changed to the one containing no serum (conditioning medium) in order to prepare the cells for stimulation. On day six in the morning, new conditioning media was added, and the plates were connected to the C-Pace EM stimulator (Ionoptix LLC, USA). Cells were stimulated with constant voltage (11.5 V, 2 ms, 1 Hz) for 24 h. Control samples that had the lid with electrodes, however, these were not connected to the stimulation device.

2.9 Lipid nanoparticles preparation

For microRNA manipulation in cell culture microRNA inhibitors, mimics and scramble microRNA controls were purchased from Qiagen, USA (Table 4). Next, they were encapsulated into manufactured lipid nanoparticles (LNPs) according to the manufacturer's protocol (Neuro9 siRNA Spark, Precision NanoSystems, Canada). Lyophilized microRNA inhibitors, mimics and controls were dissolved in storage buffer provided by the company to final concentration of 1 mM. Nucleic acids were mixed with formulation buffers so that

the final concentration was 930 μg/mL. LNPs were formulated in the manufacturer's cartridges using NanoAssembler Spark™ device (Precision NanoSystems, Canada). Prepared LNPs were later stored at 4 °C. LNPs were given to each well in a concentration of 0.01 μg/mL followed by adding 10 ng of ApoE provided in the mentioned kit.

Table 4: List of microRNA inhibitors, mimics and control

microRNA	Mature Sequence (5'-3')	Gene Globe ID
Mmu-409-5p inhibitor	AGGUUACCCGAGCAACUUUGCAU	YI04105576
Mmu-409-5p mimic	AGGUUACCCGAGCAACUUUGCAU	YM00471203
Mmu-501-3p inhibitor	AAUGCACCUGGGCAAGGGUUCA	YI04104837
Mmu-501-3p mimic	AAUGCACCCGGGCAAGGAUUUG	YI04104837
Negative control inhibitor	TAACACGTCTATACGCCCA	YI00199006-DCA
Negative control mimic	GAUGGCAUUCGAUCAGUUCUA	YM00479903

2.10 Total RNA purification

2.10.1 Total RNA purification from human blood samples

Total RNA including small RNA was manually purified from whole blood samples using PAXgene® Blood microRNA Kit (PreAnalytiX GmbH, Switzerland). Before the beginning of isolation samples were put at room temperature for 2 h. The tubes were centrifuged at 4000 × g for 10 min and supernatant was removed. After pellet resuspension in RNase-free, this step was repeated one more time. The pellet was then dissolved in a given BM1 buffer. Buffer BM2 and proteinase K were added and then the samples were incubated at 55 °C for 10 min. Samples were pipetted into lilac spin columns and centrifuged for 3 min at maximum speed. Supernatant was transferred into new tubes and iso-propanol was added. Samples were pipetted again into new red spin columns and centrifuged at 10000 × g for 1 min. The columns were then transferred into new processing tubes. Samples were washed once for 15 sec with buffer BM3 and then incubated in DNase solution for 15 min at room temperature. Spin columns were washed again, and processing tubes were changed. Buffer BM4 was added and centrifuged at 10000 × g for 2 min. Columns were dried by additional short centrifugation. Columns were placed into new tubes and buffer BM5 was applied onto

membrane. This step was repeated twice. After 1 min centrifugation at 10000 × g the tubes were incubated at 65 °C for 5 min and immediately placed on ice. Samples were then stored at -80 °C till further use.

2.10.2 Total RNA purification from mouse brain samples and neuronal cultures

Total RNA extraction including microRNA was followed by adding Tri reagent (Tri Reagent, Sigma-Aldrich, Germany) and proceeding with RNA Clean & Concentrator™ -5 Kit (Zymo Research Europe GmbH). 800 µl Tri reagent was added per CA1 brain tissue and the tissues were homogenized using a mechanical homogenizer. After homogenization, 200 µl chloroform was added and the solution was mixed using a benchtop mixer. Samples were kept at room temperature for 3 minutes. Next, samples were centrifuged for 15 min at 12000 × g at 4 °C. This high-speed centrifugation resulted in the formation of two layers: aqueous phase on top and organic phase at the bottom. For RNA isolation, the aqueous phase was carefully isolated without disturbing the interphase and mixed with 1x volume of 100 % ethanol. The solution was then transferred to zymo spin columns provided by RNA Clean & Concentrator kit and centrifuged for 1 min at 12000 × g. Columns were washed with 400 µl RNA Wash Buffer followed by incubation in DNase digestion mix (DNase I + buffer) for 15 min at room temperature. After incubation, 400 µl RNA Prep buffer was added to the column and centrifuged for 1 minute at 12000 × g. The flow through was discarded and the spin columns were washed two times with RNA wash buffer. While the first washing step involved centrifugation for 1 minute at 12000 × g, during the second wash, spin columns were centrifuged for 2 minutes at 12000 × g. To dissolve RNA, 22 µl of DNase/RNase-Free Water was carefully added on the membrane of the zymo spin column and RNA was collected in RNase free 1.5 ml tubes after centrifugation at 12000 × g for 1 min. The concentration of RNA was measured using Nanodrop™ 2000 Spectrophotometer (Thermo Fisher Scientific Inc, USA). Finally, isolated total RNA was stored at - 80 °C until further use.

2.11 Real-time quantitative PCR

First complementary DNA (cDNA) was synthesized from total RNA pool using miScript II RT Kit (Qiagen, USA) for quantitative polymerase chain reaction (qPCR). MiScript SYBR® Green PCR Kit (Qiagen, USA) was used for microRNA detection. Quantification of mature microRNAs was done on LightCycler® 480 Instrument II (Roche Diagnostics GmbH, Germany) according to the manufacturer's recommended protocol. RNU6B was used as

control for normalization of values. Relative expression was analyzed using $2^{-\Delta\Delta c(t)}$ method as described before (Livak and Schmittgen 2001). Quantitative data were plotted as mean ± standard error of mean (SEM) and unpaired parametric t-test was performed using Prism 8.3.1 program (GraphPad Software LLC, USA). All primers were purchased from Qiagen, USA (Table 5).

Table 5: List of primers used for qPCR experiment

microRNA	Mature Sequence (5'-3')	Gene Globe ID
Mmu-409-5p	AGGUUACCCGAGCAACUUUGCAU	MS00011977
Mmu-501-3p	AAUGCACCCGGGCAAGGAUUUG	MS00032928
Hsa-501-3p	AAUGCACCCGGGCAAGGAUUCU	MS00009828
Hsa-RNU6-2-11	GTGCTCGCTT CGGCAGCACA TATACTAAAA TTGGAACGAT ACAGAGAAGA TTAGCATGGC CCCTGCGCAA GGATGACACG CAAATTCGTG AAGCGTTCCA TATTTT	MS00033740

Mmu: Mus musculus, Hsa: Homo sapiens

2.12 Small RNA sequencing

NEBNext® Small RNA Library Preparation Set (Neu England Biolabs, USA) was used to generate high quality microRNAome data from human blood samples. Briefly, for the generation of microRNAome data, the total amount of 100 ng of RNA was taken for further cDNA preparation, fragmentation, adaptor ligation and hybridization. After pooling the libraries together, polyacrylamide gel electrophoresis (PAGE) was run for size selection. The insert size of 150 base pairs (bp) was chosen for further quantification and purification. Sequencing of 2 nM concentration was performed on HiSeq 2000 sequencing system (Illumina, USA) using 50 bp single read setup. Sequencing data were demultiplexed using

CASAVA v1.8 (Illumina, USA) and raw sequencing files in the format of fastq were generated.

2.13 Bioinformatic processing of microRNA sequencing data

FastQC v0.11.15 software was used for the quality control check of sequencing data (bioinformatics.babraham.ac.uk/projects/fastqc). The following parameters were analyzed: total number of reads, percentage of Guanin-Cytosine (GC) content, sequence quality per base, N content per base, sequence length distribution, duplication levels, overrepresented sequences and Kmer content. In order to identify and quantify microRNA in sequencing data the miRDeep2 package was used (Friedländer et al. 2008). Sequence retrieval for hg38/human genomes was done using UCSC genome browser (genome.ucsc.edu). The raw sequencing reads were mapped to the reference genome and microRNA counts were generated using miRDeep2 package following the developer's instructions.

2.14 Co-expression analysis of microRNA sequencing data

Weighted gene co-expression network analysis was done using WGCNA R package (v.1.61). First, microRNA counts were normalized to libraries size, followed by transformation in to log2 values. A quality z-score was calculated for each sample, and samples with low quality ($Z > 2.5$ or $Z <-2.5$) were defined as outliers and removed from further analysis. Surrogate variables were determined by sva R package (Leek et al. 2012) and effects of the covariates were adjusted using a mixed linear model. Pair-wise bi-weighted mid-correlations between microRNAs were calculated and a threshold power of 13 was chosen based on approximate scale-free topology and used to calculate pair-wise topological overlap between microRNAs in order to construct a signed network. Identification of the co-expressed modules was performed with the minimum module members of 10 and deepSplit size 3. Modules with modular eigengenes that were highly correlated were merged using mergeCloseModules function setting the dissimilarity correlation threshold at 0.15. Module membership score was set to 0.40 to further filter the microRNA members in a given module. Different modules were summarized as a network of modular eigengenes (ME) which were then correlated with the composite cognitive score calculated as explained above.

Networks of MEs were then generated for different modules and then used to correlate with available phenotypic information. Moreover, MEs were used to compare the expression of

modules between time points using a nonparametric test. MEs were correlated with the results from cognitive tests performed before and after exercise.

2.15 mRNA sequencing

TrueSeq® Library preparation kit (Illumina, USA) was used as described before (Islam et al. 2021). For that, a concentration of 500 ng of total RNA was used. The RNA quality was analyzed on 2100 Bioanalyzer Instrument (Agilent Technologies, USA). Libraries were quantified on a Qubit 2.0 Fluorometer (Life Technologies, USA). Concentration of 2 nM was used for 50 bp single end sequencing using HiSeq 2000 (Illumina, USA). BCL basecall files were translated to fastq files by bcl2fastq conversion software v.2.18.0. A quality control check of the sequencing data were performed using FastQC v0.11.15 software (bioinformatics.babraham.ac.uk/projects/fastqc/). The reads were mapped to mm10/mouse genome using using STAR aligner v2.5.2b (Dobin et al. 2013). Count files were created using featureCounts software of subread package v1.5.1.

2.16 Differential expression analysis of microRNA and mRNA

MicroRNAs with reads count of at least five in one-third of all the samples were selected for differential expression analysis. Expression data were normalized to library size and log2 transformed. Unwanted surrogate variables were determined using sva. Adjustment of the normalized expression for unwanted effect due to biological covariates (e.g. age, gender, education), and those surrogate variables was performed using a mixed linear model as described before (Islam et al. 2021). Limma R package was used to test for differential expression and de-regulated microRNAs with adjusted p value < 0.05 were considered as significantly changed.

2.17 Gene ontology and analysis of biological processes of microRNA target genes and mRNAs

To perform gene ontology analysis for microRNA targets genes, target genes were retrieved from miRTarBase database (version 8) (mirtarbase.cuhk.edu.cn/php/index.php). Gene ontology (GO) analysis on those target genes was performed using online tool (geneontology.org) and statistically significant GO terms were retrieved (false discovery rate < 0.05, Benjamin-Hochberg corrected). The significant GO terms were further filtered based on previous curation of GO annotation relevant for dementia research (from

Alzheimer's Disease Association at UCL and Alzheimer's project at University of Toronto) and similar GO terms were merged into clusters using GO semantic similarity. The parental GO term was selected for further comparative analysis and visualization. For gene ontology analysis of the deregulated mRNAs from manipulation of hippocampal neurons, a similar approach to the one described above was applied except that the step of using miRTarBase was avoided.

2.18 Immunohistochemistry

After 72 h treatment with LNPs, as described above, cell cultures that were growing on glass cover slips were fixed at DIV 10 with 4 % Paraformaldehyde (Sigma-Aldrich, Germany) for 30 min on shaker at room temperature. Later, they were quenched in 100 mM NH_4Cl solution (Merck, Germany). For synapses quantification, coverslips were then washed and permeabilized three times for 10 min and then blocked in 0.1 % Triton-X (Merck, Germany), 3 % bovine serum albumin (BSA) (AppliChem GmbH, Germany) in PBS for 1 h. Primary antibodies were then applied in 1:500 dilution for 1 h. After washing three times with PBS for 5 min, secondary antibodies were added in 1:200 dilution and incubated for 1 h. Before mounting cover slips on a glass slide, they were again washed three times using PBS. The whole procedure was done on a shaking platform at room temperature. Guinea pig Synaptophysin 1 (SySy, Germany), rabbit postsynaptic density (PSD)-95 (Cell Signaling Technology, Germany), microtubule-associated protein 2 (MAP2) (SySy, Germany) were used as primary antibodies. Corresponding secondary antibodies were donkey-anti-guinea Cy3 (Jackson Imm., UK) and goat-anti-rabbit Abberrior STAR 635p (Abberrior Instruments GmbH, Germany). For spine density analysis the coverslips were quickly washed with PBS three times. Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI) Stain (Invitrogen™, US) dye was then applied. Five to six crystals were added on top of the coverslips and 200 µl of PBS was added. Total incubation time was 10 min on a benchtop shaker at room temperature. Afterwards, the coverslips were washed with PBS until all the crystals were no longer visible. The plates with 1 mL of PBS were placed on a shaker at 4 °C overnight. After this long washing step, the coverslips were washed again three more times and mounted using Fluoromount-G™ (Invitrogen™, Germany).

2.19 STED microscopy and image analysis

Stimulated Emission Depletion (STED) microscope Leica TCS SP5 (Abberrior Instruments GmbH, Germany) with the 100x oil-immersion objective and photomultiplier signal detector

was used for acquiring high resolution images of Synaptophysin 1, PSD-95 and MAP2 stained primary neuronal cultures. The areas of interest were defined randomly and the images were obtained in one plane using settings recommended for the chosen secondary antibodies. Analysis of colocalization of pre- and post-synaptic markers was performed using SynQuant plugina in ImageJ v. 2.0.0-rc-69/1.52p.

For DiI stained coverslips STEDYCON system installed on Leica microscope DMi8 with 63x oil-immersion objective was used. Default parameters for Cy3 wavelength were chosen. The whole stack images were obtained. Z-stack was defined automatically by STEDYCON smart control software. Stack images were later merged in ImageJ v.1.8.0_202. Spine density on a given dendritic length was defined manually using NeuronJ plugin. The data were plotted and statistical analysis using parametric t-test was performed using Prism 8.3.1 program (GraphPad Software LLC, USA).

2.20 Multi-electrode assay

E17 hippocampal regions were extracted as described above. Cells were centrifuged with the speed 300 × g for 5 min at room temperature and resuspended in NbActiv4® neuronal culture medium (BrainBits, USA). Cells were counted, mixed with 1 μg/mL laminin (Merck, Germany) and plated at density of 15000 cells/μL into Lumos lens lid 48 well microelectrode array (MEA) plates equipped with 16 electrodes in each well. These wells were coated with 0.05 mg/L PDL in advance. 30 % of media change was conducted every third day. At DIV 7, spontaneous basal activity was recorded. Afterwards cells were treated with LNPs containing micro-RNA inhibitors mix or scramble RNA as control in the same concentration as described above. Starting from DIV 10, spontaneous neuronal activity was recorded using the Maestro Apex Platform (Axion Biosystems, USA). Every 3 h during 24 h the measurements were recorded for 10 min. The data were later extracted and analyzed using neuronal module AxlS Navigator software (Axion Biosystems, USA). All the values were plotted and analyzed using Prism 8.3.1 program (GraphPad Software LLC, USA).

2.21 Human samples

Postmortem human brain tissues from patients with diagnosed genetic frontotemporal dementia were obtained under a material transfer agreement from the Netherlands Brain Bank. Several samples were received from the Queen Square Brain Bank of Neurological Disorders and Medical Research Council, King College London. This project is a part of

„The risk and modifying factors in frontotemporal dementia (RiMod-FTD) consortium“. The goal of this consortium is to create a multi-model and multi-omics data resource focusing on mutations in the three following genes: microtubule associated protein tau (MAPT), progranulin (GRN), and chromosome 9 open reading frame 72 (C9orf72 or C9) (DESGESCO et al. 2020). In total we received samples from 23 patients (8 men and 15 woman) whose mean age was 63.5 ± 7.2 with a history of frontotemporal dementia. Among them eleven patients had MAPT, seven had C9orf72 and five GRN mutation. We also received postmortem brain samples from 13 people (4 men and 9 women) with the mean age of 80.4 ± 8.3 years with no history of frontotemporal or any other type of dementia. All tissues were part of the temporal cortex.

Brain samples from the frontal cortex were collected from Massachusetts General Hospital, USA. The samples were obtained from patients who participated in a brain donation program (Au et al. 2012). They were classified according to neurofibrillary changes and their localization based on Braak staging. Six stages can be further classified into three stages which share similar characteristics. Stages I-II are characterized mainly by pathology in transentorhinal and entorhinal regions. At stages III-IV the lesions area is extended to neocortical areas of lingual gyrus, fusiform gyrus and association areas. These stages also show a much higher density of lesions in comparison to first two stages. Finally, at stages V-VI most of the cortical regions are affected and layered structure is hardly recognizable (Braak et al. 2006). In total 30 postmortem samples were collected from patients with diagnosed Alzheimer’s disease (12 men and 18 women). Among them nine patients had stages I-II, eleven had stages III-IV and ten had stages V-VI.

2.22 Statistical analysis

Statistical testing for sequencing analysis of sequencing experiments is described in the corresponding section and in the figure legend. As for other experiments the analysis was performed using Prism 8.3.1 program (GraphPad Software LLC, USA). The results from TMT-A, TMT-B, WCST and VLMT were assessed using Wilcoxon paired nonparametric t-test. In all the tests $p < 0.05$ was considered as significant. For wet lab experiments the data were first checked for normal distribution. The control group and the group treated with microRNAs groups were compared with two-sided unpaired parametric Student’s t-test. For analysis of human dementia samples ordinary one-way analysis of variance (ANOVA) test with Dunnett’s or Turkey’s multiple comparison was performed. Kruskal-Wallis test with multiple comparison was used to compare electrical activity of neurons in MEA experiments.

The level of significance was defined as $p < 0.05$. All final images with graphs were created using BioRender.com.

3 Results

3.1 Identification of circulating microRNAs associated with cognition in healthy volunteers

To identify circulating microRNA that can potentially play a role in cognitive function participants with no history of cognitive impairment were recruited. Healthy volunteers were participating in a three-month aerobic training during which the cognitive scores and whole blood samples before the very first and right after the last session were obtained and analyzed (Figure 3A).

To assess cognitive function and their potentional improvement after physical exercise neurophysiological tests that are meant to evaluate visual attention, mental flexibility and memory were performed. The speed of processing the task was compared before and after training using the data obtained from TMT-A and TMT-B cognitive testing, the executive function was measured with WCST, memory was estimated using VLMT. No significant difference in the amount of time needed to solve the task can be seen in TMT-A and TMT-B (Figure 3B and 3C). For WCST, the performance was better when compared to the time point before exercise. The total number of trials administrated and the total number of errors as well were reduced after the training period (Figure 3D and 3E). No significant difference could be seen in STM after regular training (Figure 3F). LTM after regular aerobic exercise was significantly better than before starting exercising. The total number of the words recalled for both tests was increased. (Figure 3G).

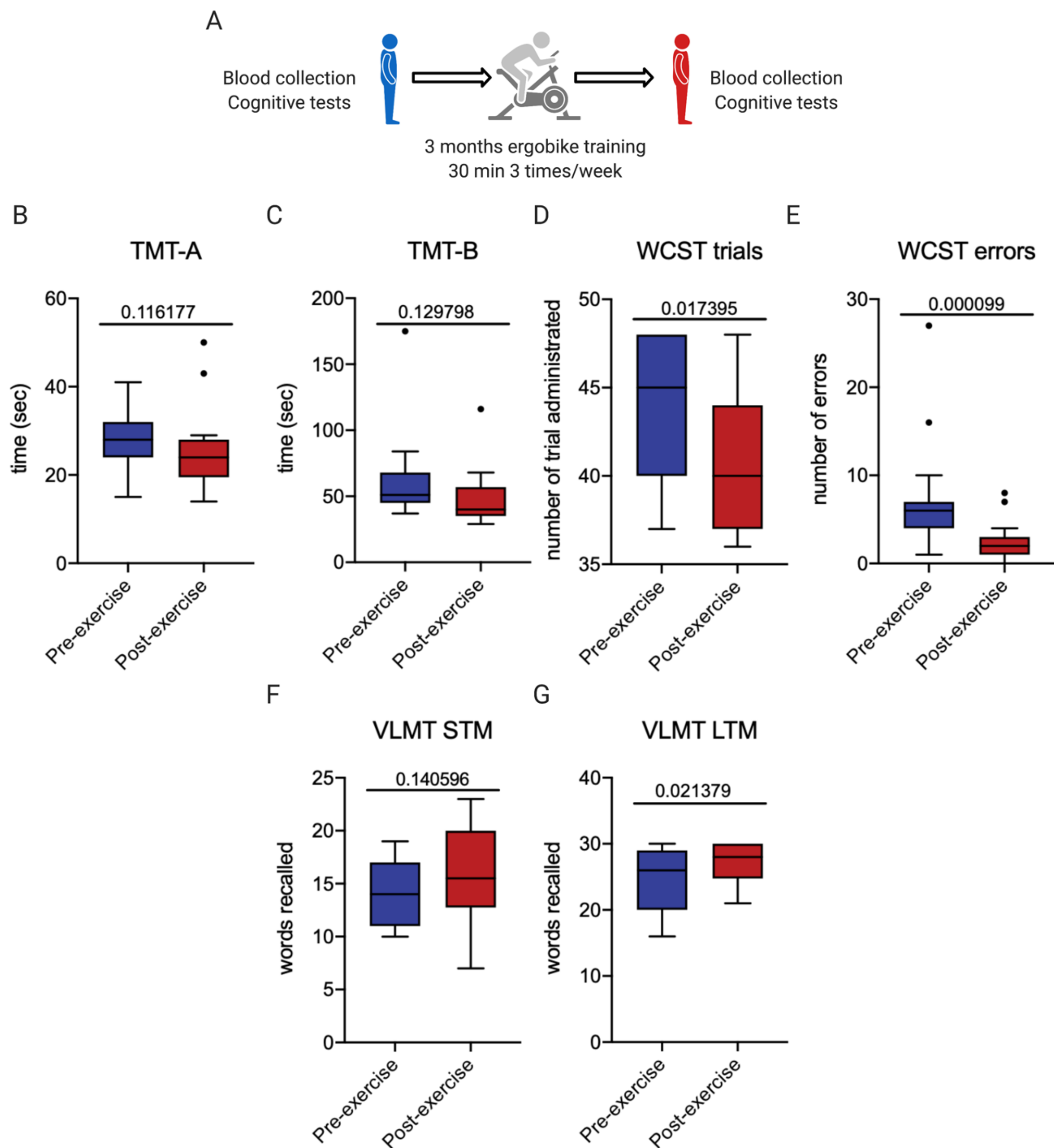

Figure 3: Human exercise paradigm and cognitive testing. (A) Blood was collected, and cognitive tests were performed in healthy male (n = 14) and female (n = 5) subjects before and after three-month ergometer training for 30 min three times a week. Time in seconds spent on (B) Trail Making Test A (TMT-A) and (C) Trail Making Test B (TMT-B) before and after exercise. (D) Total number of trials administrated and (E) total number of errors made before and after exercise in Wisconsin Card Sorting Test (WCST). Number of words recalled as estimation of (F) short-term memory (STM) and (G) long-term memory (LTM) from Verbal Learning Memory Test (VLMT). The values are presented as Turkey whiskers boxplot with outliers depicted as separate data points. Paired nonparametric Wilcoxon matched pairs signed rank test was used, $p < 0.05$ was considered significant.

Next, small RNA sequencing of blood total RNA was performed from samples collected before and after exercise to detect circulating microRNA that could be linked to improved cognitive functions. Weighted microRNA co-expression analysis was performed with the goal to identify different expression modules of microRNAs. In total, 33 modules of microRNAs were found, among those, 5 modules were deregulated with pre- and post-exercise, but not with other parameters such as age, sex, years of education or smoking status. Interestingly, midnightblue module displayed most deregulation and its expression was increased after exercise (Figure 4A and 4B). Further analysis was aimed to see if these modules could be correlated with the results from cognitive tests performed at the beginning and end of the experimental design. Midnightblue module was positively correlated to LTM, suggesting that the individuals with increased expression of midnightblue module in their blood displayed better LTM. Similar correlation was observed for WCST, a test that is widely used to evaluate cognitive flexibility. Between TMT-A and TMT-B that participants performed in the study, the midnightblue module showed negative correlation to performance in TMT-A test at p value 0.09, whereas this correlation to the performance in TMT-B test was highly significant ($p = 0.04$) (Figure 4C). This result suggests that the increased expression of midnightblue module of microRNAs was related to decreased time required to finish the given tasks in trail making tests, particularly the task that required cognitive flexibility. All together these data suggest that expression of midnightblue is linked to better cognitive performance after regular aerobic training. When the module microRNA members of midnightblue cluster were investigated, 18 microRNAs were identified. An intramodular network connectivity of the module microRNAs based on intramodular correlation was built in Cytoscape (v 3.6.1). Figure 4D displays the microRNA members of midnightblue module and their intramodular connectivity. Gene ontology analyses based on the 18 microRNA members in midnightblue module revealed interesting biological processes, including learning or memory, warranting that their roles in neuronal plasticity should be experimentally investigated (Figure 4E).

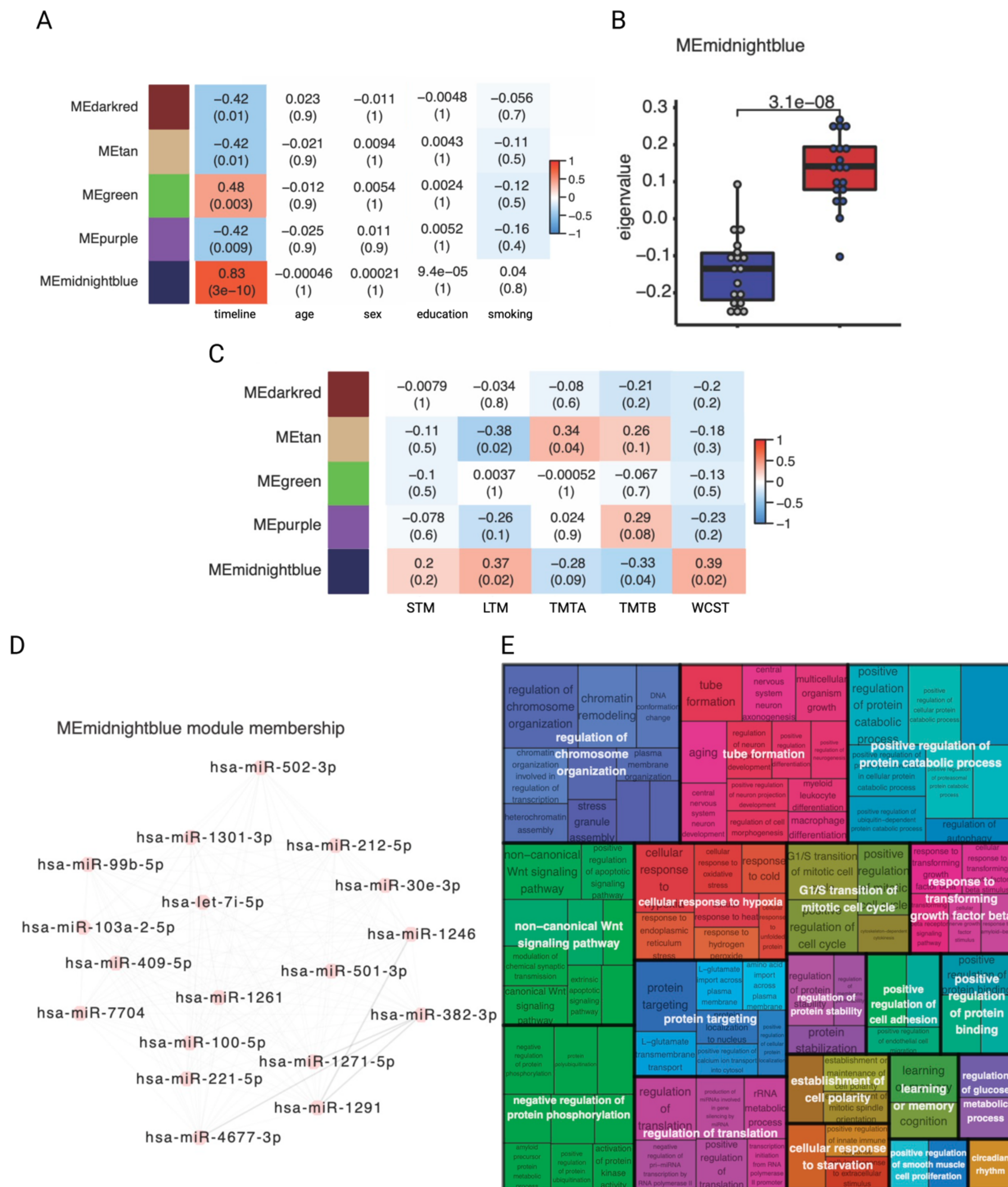

Figure 4: Weighted microRNA co-expression analysis and correlation with clinical traits and cognitive performance. (A) A heatmap chart showing correlation between the five most deregulated module eigengenes (ME) and clinical traits. Each row represents a ME, each column corresponds to a trait. Each cell shows the corresponding correlation, p values are shown in brackets ($p < 0.05$ was considered significant). The values are color-coded based on direction and degree of correlation. Blue represents negative correlation, red represents positive correlation. (B) Comparison of eigenvalues from pre- and post-exercise conditions of ME midnightblue. Wilcoxon t-test was used. (C) A heatmap chart showing correlation between ME expression and cognitive performance based on following tests: short-term memory (STM) and long-term memory (LTM) scores from Verbal Learning Memory Test (WLMT), speed of solving Trail Making Test A and B (TMT-A and TMT-B), score of Wisconsin Card Sorting Test (WCST). Each cell shows the corresponding correlation, p values are shown in brackets. The values are color-coded as described above. (D) MicroRNA network of ME midnightblue representing 18 top hub microRNAs and their interconnectivity. (E) Gene ontology (GO) terms

of biological processes regulated by deregulated microRNAs from ME midnightblue. Pathways are presented based on their common functions and paternal terms (depicted as overlayed in white).

To refine the microRNAs as potential candidates for further experimentation, an independent differential expression analysis was performed to evaluate the expression pattern of individual microRNAs between two conditions. After rigorous statistical analyses, 26 microRNAs were found to be deregulated after exercise compared to pre-exercise time point (Figure 5A). Interestingly, 12 microRNAs overlapped with the midnightblue cluster described above (Figure 5B). Among these 12 microRNAs, the top three upregulated microRNAs were microRNA-1271-5p, microRNA-409-5p and microRNA-501-3p. We asked whether these given microRNAs are expressed in primary hippocampal neurons. While expression of microRNA-1271-5p could not be detected in primary hippocampal neurons, expression of microRNA-409-5p and microRNA-501-3p was reliably and reproducibly detected. Additionally, we performed an extensive literature review on most recent research articles (Table1 and 2). Based on these above-mentioned criteria and findings, two microRNA candidates namely microRNA 409-5p and microRNA 501-3p were chosen for further experiments.

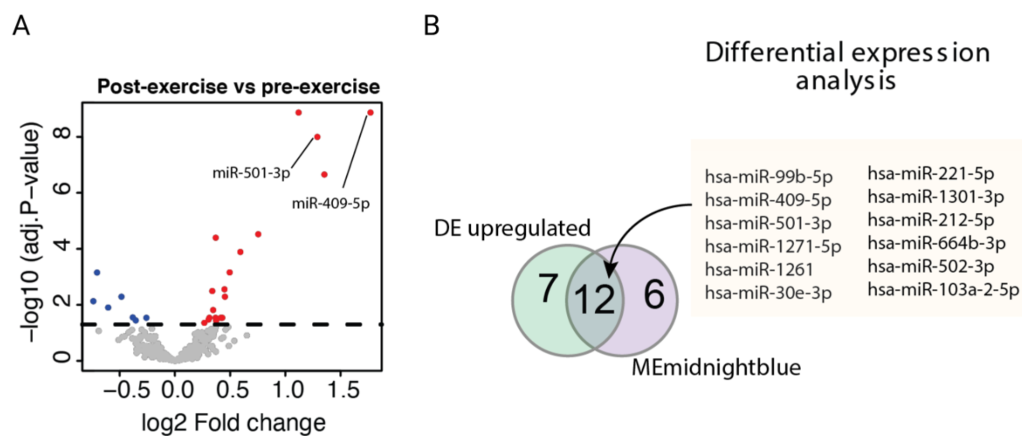

Figure 5: Differentially expressed microRNAs pre- and post-exercise. (A) Volcano plot of differentially expressed microRNAs between samples collected before and after exercise. The x-axis shows differential expression log2, blue represents downregulation, red represents upregulation. The y-axis shows adjusted p value -log10 for differential expression. Each dot represents one single microRNA. Values above the line are considered significantly deregulated with adjusted p value < 0.05. (B) Venn diagram representing common genes between differentially upregulated genes and top hub genes of midnightblue module.

3.2 Identification of selected microRNAs in experimental exercise model

Since we observed an increased expression of these two microRNAs in blood after exercise, we asked if exercise could induce similar changes in skeletal muscle. To this end, we first cultured and differentiated commercially available mouse myoblast cell line C2C12. After 5 days of differentiation, myotubes displayed spontaneous contraction and relaxation. Next, the differentiated myotubes were electrically stimulated for 24 h to model exercise *in vitro*. Total RNA from cells was then purified and analyzed with qPCR (Figure 6A). Typical longitudinal shape (Figure 6B) and spontaneous contractions could be seen prior stimulation. Despite high variability in expression (6.114 ± 2.681 for microRNA-409-5p and 5.052 ± 2.133 for microRNA-501-3p), the significant upregulation of both microRNA-409-5p and microRNA-501-3p could be observed (Figure 6C and 6D).

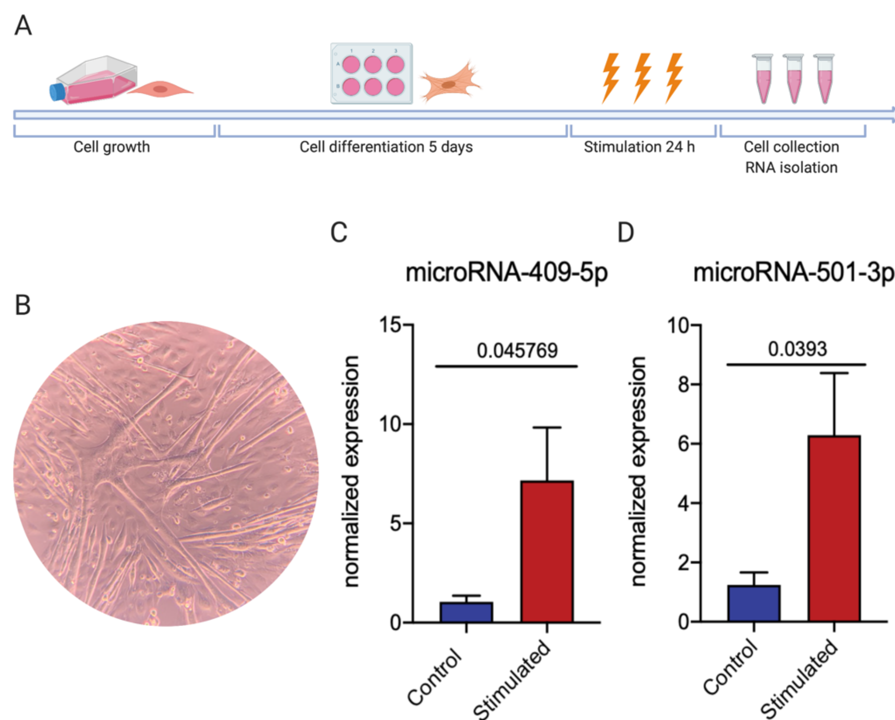

Figure 6: Upregulation of microRNAs in muscle cells after electrical stimulation. (A) Experimental design. Myoblast cell lines were first cultured and then differentiated into mature muscle cells. At fifth day of differentiation electrical stimulation for 24 h was applied and the cells were collected. (B) Picture of mature muscle cells in typical longitudinal shape prior to stimulation. Quantification of (C) microRNA-409-5p and (D) microRNA-501-3p expression levels with qPCR in stimulated murine muscle cells (n = 6/6) in comparison to non-stimulated controls (n = 6). Bar charts show normalized expression relative to control, data shown as means ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

Since we observed cognitive enhancement in individuals performing exercise, we asked if the two microRNAs could increase in expression in the central nervous system after physical exercise. To investigate this, we tested the expression of these microRNAs in brain samples from a mouse model of exercise that was previously done in the lab. In this experiment, the previous experimenter subjected 20 mice to two different conditions. One group (runners) had constant access to a functioning running wheel (n = 10), another group (sedentary) also had wheels in the cages but those were blocked so running was not possible (n = 10). The mice were in the experiment for 18 weeks (Figure 7A). Cognitive tests performed on these mice revealed cognitive enhancement in runners compared to sedentary (unpublished data). Using the samples from the same mice, we asked whether these two microRNAs could change in expression in the hippocampus between sedentary and runners. To this end, we isolated RNA from already dissected hippocampal subregions and performed qPCR for microRNA expression. Two samples were found as outliers using statistical measures, hence were removed for further analysis. Interestingly, both microRNA-409-5p (Figure 7B) and microRNA-501-3p (Figure 7C) were upregulated in the hippocampi of runners (n = 9) when compared to those from sedentary (n = 9).

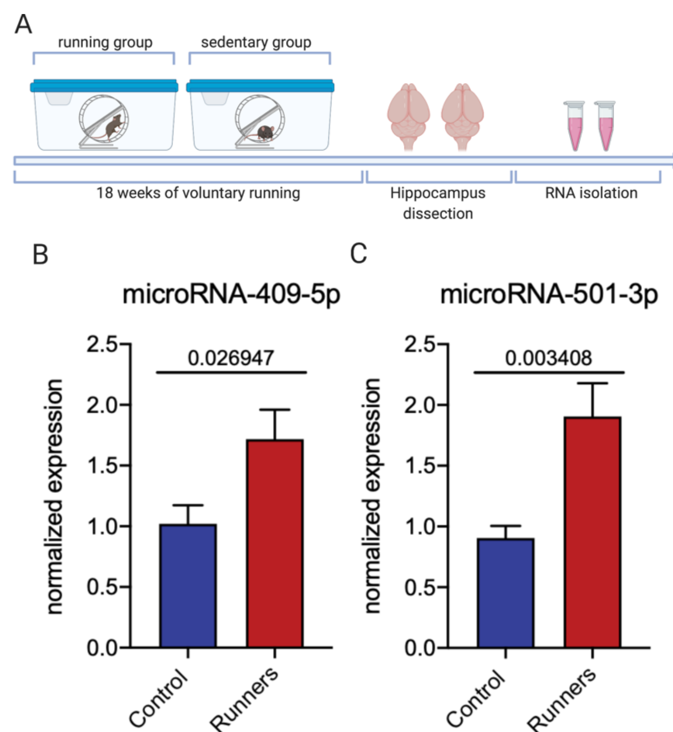

Figure 7: Increased expression of microRNA in mouse exercise model. (A) Experimental design of exercising mice. Mice from experimental group (n = 9) had free access to functioning wheel for voluntary running during the whole period of 18 weeks. Mice from control group (n = 9) had wheels blocked in their cages. The experiment went on for 18 weeks. Quantification of (B) microRNA-409-5p and (C) microRNA-

501-3p qPCR expression levels in hippocampal region. Bar charts show normalized expression relative to control, data shown as means ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

3.3 Inhibition of microRNAs alters neuronal plasticity

3.3.1 Genes differential expression in response to microRNA inhibition

Based on the observation that our two candidate microRNAs increase in expression in the hippocampi of mice after exercise, we decided to manipulate these microRNAs to assess their effect at the functional level. To this end, we employed various experimental designs *in vitro* using primary murine neuronal culture. First, freshly prepared LNPs packaged with inhibitors of microRNA-409-5p (anti-miR-409-5p) and microRNA-501-3p (anti-miR-501-3p) were applied to primary hippocampal neurons at DIV 10 and incubated for 48 hours (Figure 8A). As control, scramble microRNA packaged into LNPs was used and neurons were treated similarly. An exemplary image of neurons at DIV 10 is displayed in Figure 8B. Total RNA was isolated, and cDNA prepared in order to first confirm the inhibition of two microRNAs by qPCR. Indeed, for both microRNAs, a decreased expression was observed in cells treated with anti-miRs compared to those treated with scramble control small RNA (Figure 8C and 8D). This RNA was then used to perform RNA sequencing and differential expression analysis. More than 2000 genes were found to be significantly deregulated after inhibiting these microRNAs (Figure 8E and 8F). For a better understanding of the physiological role of all these genes, we performed gene ontology analysis. Interestingly, many of the differentially expressed genes were involved in neuronal processes such as dendrite morphogenesis, synapse maturation, regulation of neuronal death and memory (Figure 8G and 8H).

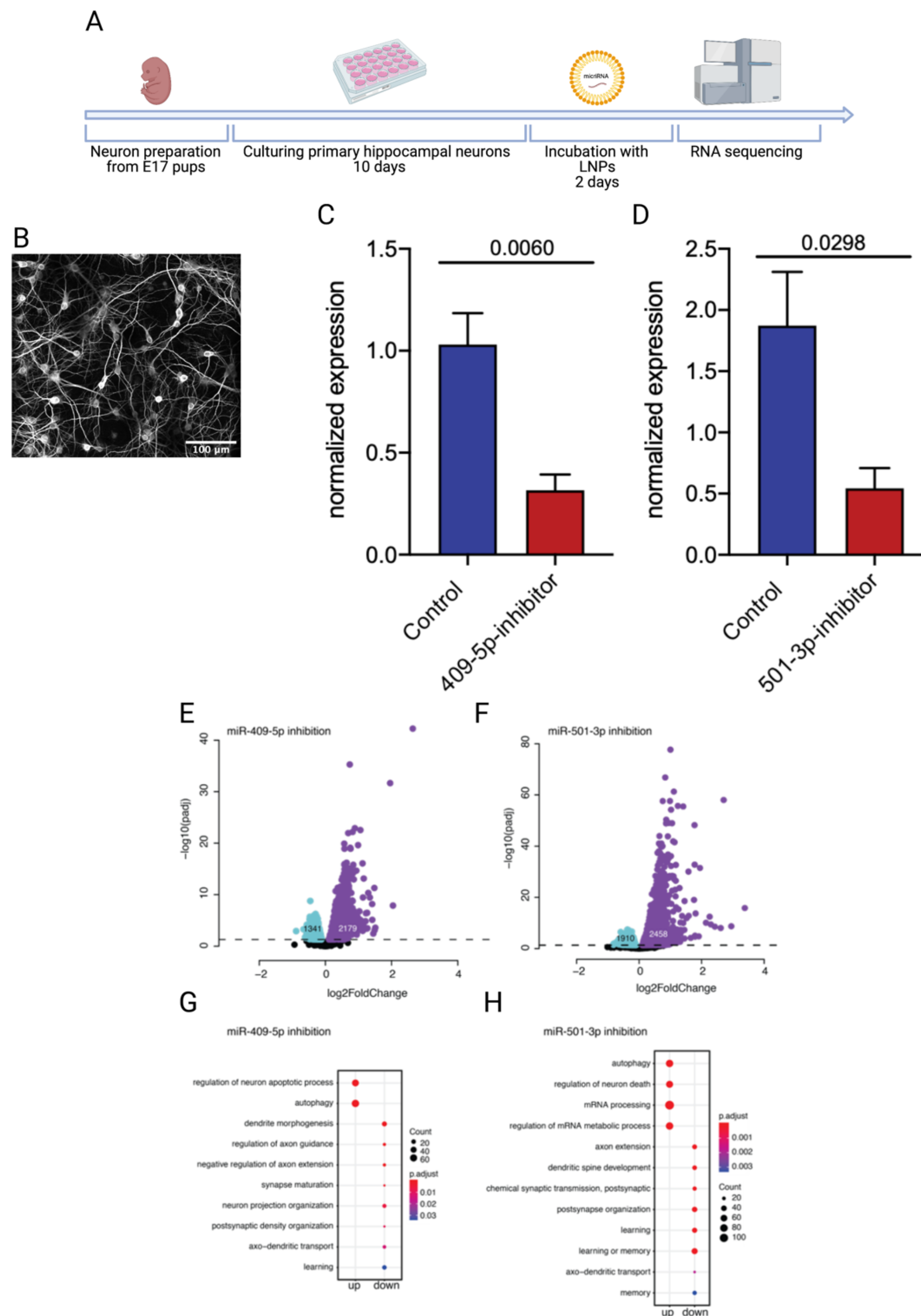

Figure 8: Inhibition of microRNA in neuronal culture reveals changes in transcriptome. (A) Experimental design of microRNA inhibition. Primary hippocampal neurons were prepared and cultured for ten days. These were then incubated with lipid nanoparticles (LNPs) for 48 hours and RNA was isolated for sequencing. (B) Example image of primary hippocampal neurons at day ten. Quantification of (C) microRNA-409-5p and (D) MicroRNA-501-3p expression by qPCR after applying inhibitors. Bar charts show normalized expression relative to control, data shown as means ± SEM, unpaired nonparametric t-test was used, $p < 0.05$ was considered significant. Volcano plots represent differentially expressed genes after RNA sequencing of neuronal cultures treated with (E) microRNA-409-5p and (F) microRNA-501-3p inhibitors (n = 6/6) when compared to treated with scramble microRNA control (n =6). The x-axis shows differential expression log2, blue represents downregulation, red represents upregulation. The y-axis shows adjusted p value -log10 for differential expression. Each dot represents one single gene. Values above the line are considered significantly deregulated with false discovery rate < 0.05. Gene ontology and pathway analysis of the predicted targets from inhibiting (G) microRNA-409-5p and (H) microRNA-501-3p reveals several potential pathways involved in

neuronal plasticity. Dots on the left represent upregulated and on the right downregulated genes. Size of the dots represents number of genes. Color intensity represents adjusted p value.

3.3.2 Inhibition of microRNAs alters neuronal morphology

The gene ontology analyses revealed that the two microRNA candidates could be involved in downregulation of axo-dendritic transport, post-synaptic organization and maturation among other important neuronal processes (Figure 8). We hypothesized that the two microRNAs might be critical for maintenance of dendritic morphology, synaptic maturation and inhibition of these microRNAs might lead to aberrant dendritic morphology and synaptic maturation. To test the effect of inhibition of these two microRNAs on dendritic morphology, we devised an experimental plan as outlined in Figure 9A. In summary, we cultured primary hippocampal neurons and treated them with LNPs containing anti-miRs and scramble control at DIV 7. Hippocampal neurons already display excitatory synapses at DIV 7 (Grabrucker et al. 2009) and DIV 7 could be an ideal time point for pharmacological interventions. After 72 h of incubation, cells were fixed and stained for further examination and visualization of fine dendritic branches.

In addition to the regular control with scramble small RNA containing LNP (scramble control), we used a no-treatment control group, where no LNP was added to the neurons (no treatment control). For dendritic spine quantification DiI staining was performed, and confocal images were taken for the anti-miR treated and control samples. Several dendrites were randomly selected from neurons and spines were then manually quantified (Figure 9B). First, we compared dendritic spine morphology between scramble control and no treatment control, and no significant difference could be detected (Figure 9C).

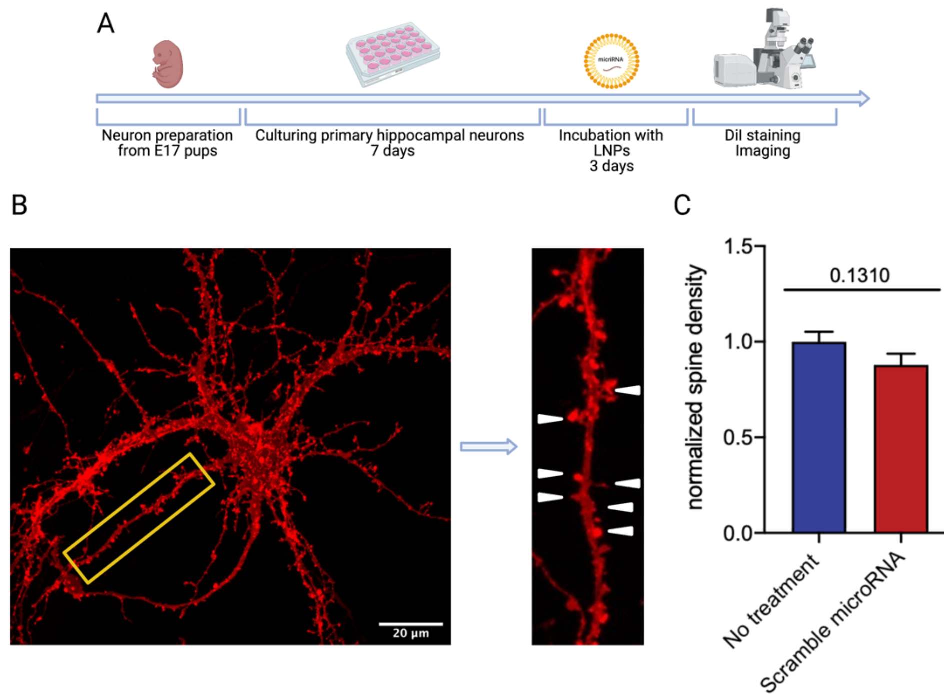

Figure 9: Spine density analysis. (A) Experimental design. Primary hippocampal neurons were cultured for seven days and then incubated with lipid nanoparticles (LNPs) for three days. Cover slips with neurons were then fixed and stained. Confocal images on STEDYCON system with 63x objective were taken. (B) Example confocal images of DiI-labeled dendritic segments with dendritic spines of primary hippocampal neuron treated with scramble microRNA. (C) Spine density was compared between treated with scramble microRNA and no treatment at all (n = 3/3). Y-axis shows number of total spines divided per length of a chosen dendritic segment, data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

We then analyzed spine density in neurons treated with microRNA inhibitors compared to controls treated with scramble microRNA (Figure 10A). Quantification analysis showed that neurons that were treated with anit-miR-409-5p displayed ~50% reduced spine density when compared to scramble control (Figure 10B). Similar reduction in spine morphology was observed for neurons treated with anit-miR-501-3p (Figure 10C). These data suggest that a reduced level of microRNA-409-5p and microRNA-501-3p can negatively affect spine morphology.

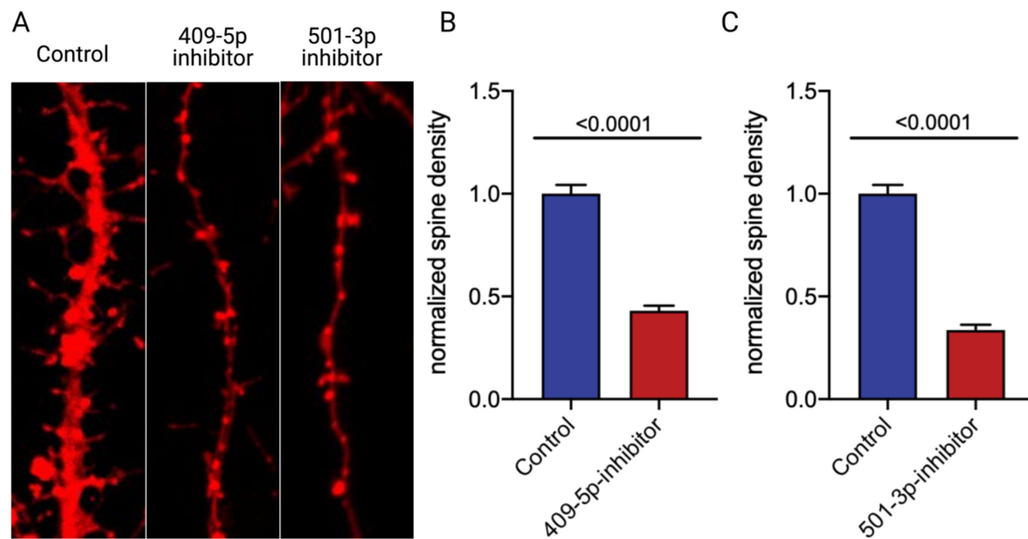

Figure 10: Altered spine density after microRNA inhibition. (A) Representative confocal images of dendrites from control and microRNA inhibitors. Spine density in (B) anti-miR-409-5p and (C) anti-miR-501-3p treated neurons (n = 6/6) in comparison to control (n = 7). Y-axis shows number of total spines divided per length of a chosen dendritic segment, data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

Dendritic spines serve as a necessary substrate for postsynaptic density and synapse formation. However, spine number does not allow the measurement of the total number of mature synapses. To test the effect of the inhibition of the two microRNAs on synaptic maturation, we devised the experiment as outlined in Figure 11A. The experimental scheme was the same as the previously described experiment (Figure 9). However, the number of mature synapses was assessed by co-localization of presynaptic density protein Synaptophysin 1 and postsynaptic density protein PSD-95 (Figure 11B). Additionally, microtubule marker MAP2 was used to label neurites (Figure 11B). Images were acquired from the random position of the coverslips. Similar to DiI staining, first neurons treated with scramble microRNA were compared to neurons that received no treatment. In line with the previous observation, no difference could be identified (Figure 11C).

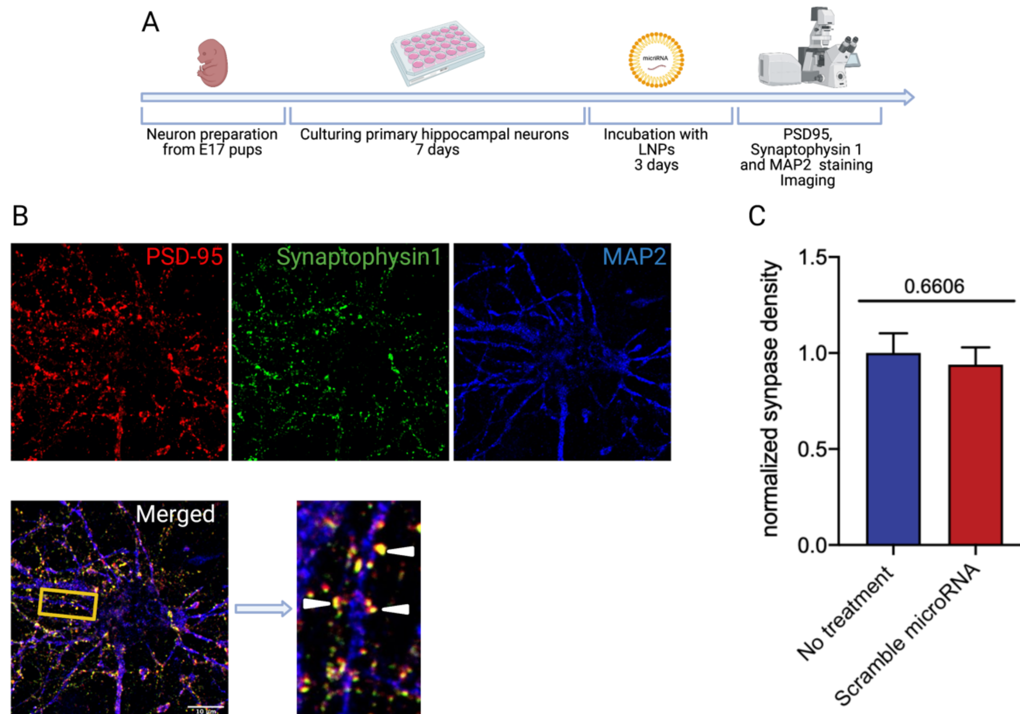

Figure 11: Synapse density analysis. (A) Experimental design. Primary hippocampal neurons were cultured for seven days and then incubated with lipid nanoparticles (LNPs) for three days. Cover slips with neurons were then fixed and stained. Confocal images were taken on STED microscope system with 100x objective. (B) Example STED images of PSD-95 in red, Synaptophysin 1 in green, and MAP2 in blue staining of primary neuronal hippocampal neurons. Colocalized signals are shown in yellow. (C) Synapse density was compared between scramble microRNA and no treatment at all (n = 3/3). Y-axis synapse density as percentage of colocalized puncta per image normalized to control, data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

Figure 12A highlights the co-localization of Synaptophyin-1 and PSD95 from scramble control, anti-miR-409-5p and anti-miR-501-3p groups. Number of co-localized puncta (mature synapse number) in the treatment groups (anti-miR-409-5p and anti-miR-501-3p) were further normalized to those from scramble control treated neurons. Analyses revealed that inhibition of microRNA-409-5p (anti-miR-409-5p) in neurons led to a significant reduction in the number of functional synapses compared to those from scramble control treatment (Figure 12B). Similar results were observed for anti-miR-501-3p treated neurons (Figure 12C)

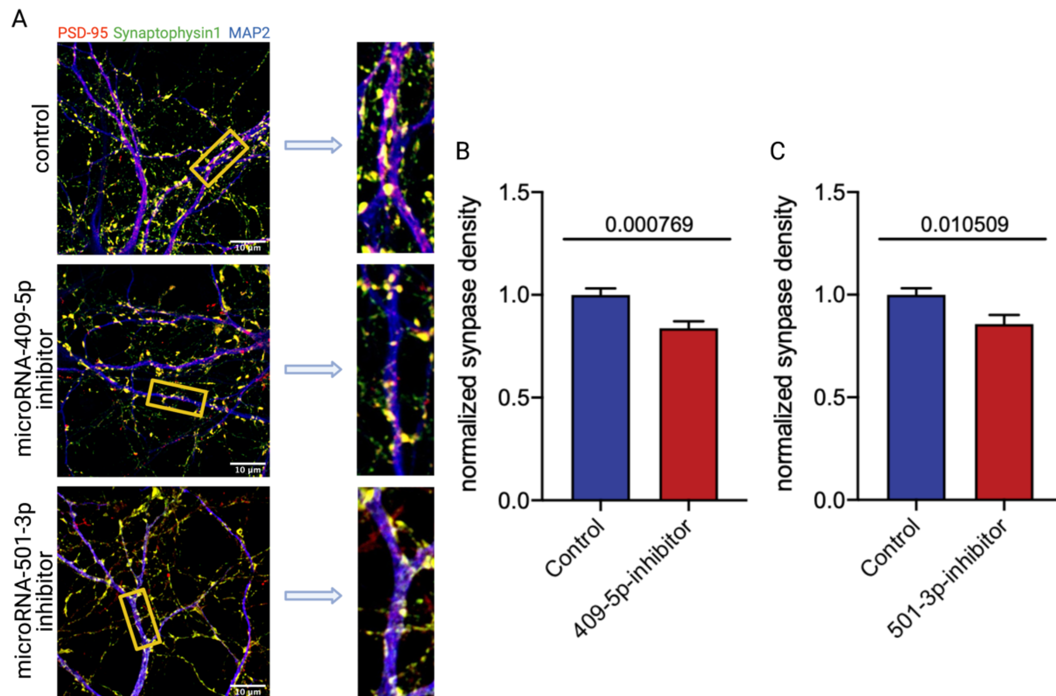

Figure 12: Reduced synapse density after microRNA inhibition. (A) Representative images of control samples and samples treated with microRNA inhibitors with three channels for Synaptophysin 1, PSD-95 and MAP2 merged together. Synapse density in (B) anti-miR-409-5p and (C) anti-miR-501-3p treated neurons (n = 6/6) in comparison to control (n = 6). Y-axis shows synapse density as percentage of colocalized puncta per image normalized to control, data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

3.3.3 MicroRNA inhibition affects spontaneous network activity

Reduced synapses and aberrant dendritic morphology are likely to affect neuronal activity and its synchrony. To test this, we performed an electrophysiology-based MEA experiment in order to assess spontaneous neuronal activity. To this end, primary hippocampal neurons were cultured in wells equipped with electrodes. At DIV 7 basal activity was first measured and then the cultures were treated with LNPs as described before. After 72 h incubation the plate was transferred to an MEA device for further recording (Figure 13A). Because we saw similar biological processes in the gene ontology analysis and a similar effect of both microRNA inhibition, we decided to use them together as a cocktail in this experiment. Anti-miR-501-3p and anti-miR-409-5p treatment led to significant reduction in neuronal weighted mean firing rate (Figure 13D), number of bursts (Figure 13E) and number of network bursts (Figure 13F).

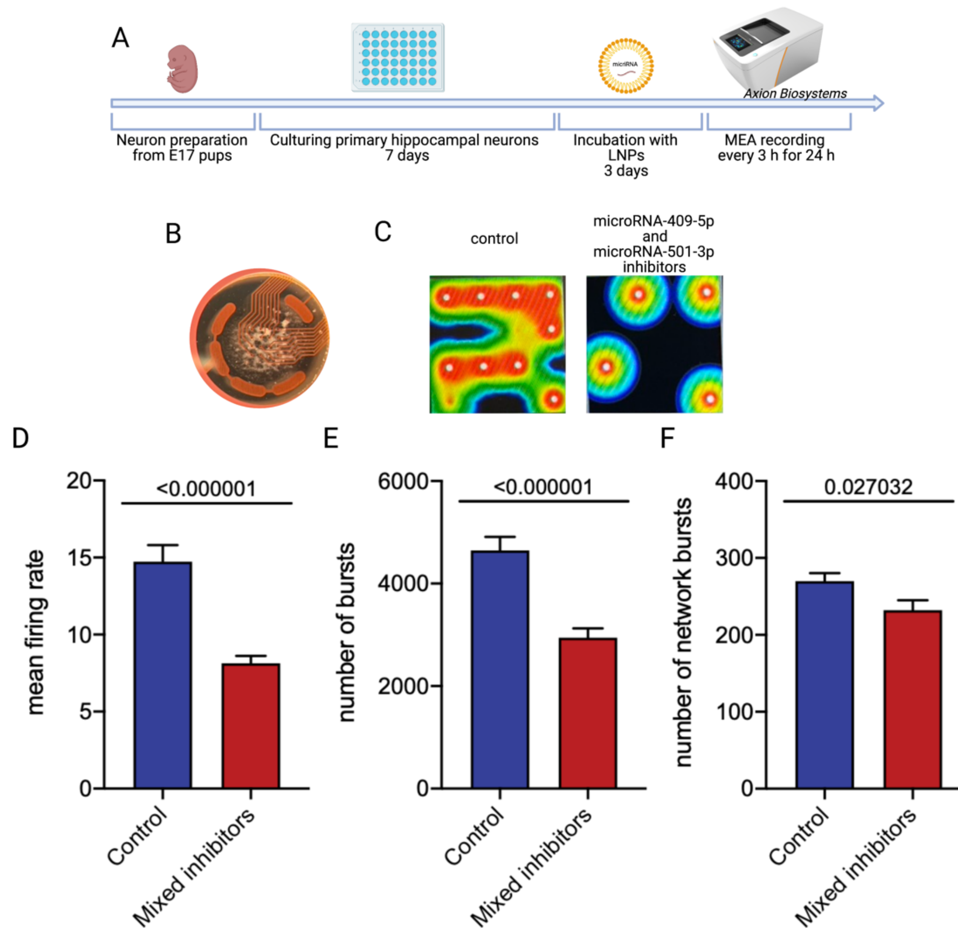

Figure 13: Reduced neuronal activity after microRNA inhibition. (A) Experimental design of electrical recordings. Cells were cultured and treated with lipid nanoparticles (LNPs) containing anti-miR-409-5p, anti-miR-501-3p (n =6/6) and scramble microRNA (n = 6) at DIV 7. The electrode field is formed by 16 electrodes. Action potentials that induce a change of potential in the immediate vicinity to the electrodes were recorded. Recordings were done every 3 h for 10 min during the period of 24 h. (B) An example image taken of neuronal culture at DIV 7. (C) Examples images of firing activity during recoding of control and inhibited neurons. (D) Mean firing rate after treatment with anti-miR-409-5p and anti-miR-501-3p. (E) Number of bursts after treatment with anti-miR-409-5p and anti-miR-501-3p. (F) Number of spontaneous network-wide synchronized bursts after treatment with anti-miR-409-5p and anti-miR-501-3p. Data are presented as mean ± SEM for each time point. unpaired parametric t-test was performed, $p < 0.05$ was considered significant.

3.4 Expression of microRNA in dementia

As we were strongly convinced that the selected microRNA candidates play a role in neuronal function we decided to investigate if their expression changes in disease conditions in patients. For that, we performed RNA isolation from postmortem human brain samples who had a medical history of frontotemporal dementia and Alzheimer's disease. Samples from patients with genetic frontotemporal dementia were characterized based on the presence of one of the most common mutations in three genes: C9, GRN or MAPT. Brain tissue samples from healthy individuals of a similar age were collected in the same study. Samples from patients with Alzheimer's disease were characterized by the widely used Braak staging method. These samples were divided into three groups: stages I-II, stages III-IV and stages V-VI. Quantification of microRNA expression was performed using qPCR. An interesting finding was observed for microRNA-409-5p in frontotemporal dementia. Its expression was significantly downregulated in all three mutations of frontotemporal dementia when compared to non-demented controls (Figure 14A). However, no significant difference was found for microRNA-501-3p (Figure 14B). As for Alzheimer's disease, a strong trend of reduced expression of microRNA-409-5p was observed, but non-significant at later Braak stages (stage III-IV and stage V-VI) compared to earlier Braak stage (stage I-II) (Figure 14C). For microRNA-501-3p, no difference was observed among different Braak stages (Figure 14D).

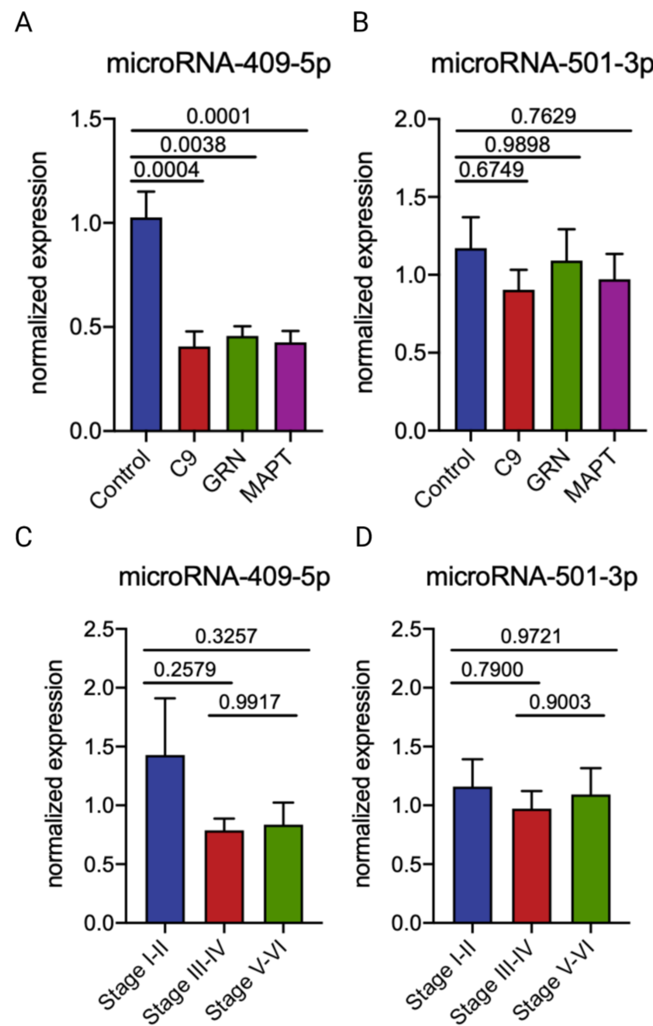

Figure 14. MicroRNA expression in dementia. Expression of (A) microRNA-409-5p and (B) microRNA-501-3p in frontotemporal dementia samples with C9, GRN and MAPT mutations (n = 7/5/11) normalized to healthy controls (n = 13). Expression of (C) microRNA-409-5p and (D) microRNA-501-3p in Alzheimer's disease in Braak stages I-II, III-IV and V-VI (n = 9/11/10). Bar charts show normalized expression presented as means ± SEM, one-way ANOVA was used, for frontotemporal dementia Dunnett's multiple comparison to control was used, for Alzheimer's disease Turkey's multiple comparison test was used, $p < 0.05$ was considered significant.

3.5 Mimicking the effect of exercise-related microRNAs in vitro

As we initially set a goal to identify microRNAs that can serve as potential target therapeutics in cognitive impairment treatment, we performed several experiments with a view to overexpress our selected candidates. Based on previous observation that the selected two microRNAs are upregulated in cultured muscle cells upon 24 h long stimulation (Figure 6), we hypothesized that transplantation of media from stimulated cells containing microRNAs might alter neuronal morphology and affect the number of spines and functional synapses.

To this end, we performed a pilot experiment where we incubated primary hippocampal neuronal cultures at DIV 7 with conditioning media (serum free) collected from stimulated muscles. As control, we collected conditioning media (serum free) from non-stimulated muscle cultures (Figure 15A) from the same experimental setting and applied it on neurons as mentioned above. Interestingly, analyses revealed an increased spine density (Figure 15B) and synapse number (Figure 15C) in neurons treated with serum free conditioning media from stimulated muscle cells compared to those treated with serum free conditioning media from non-stimulated muscle cells. These results suggest that the media from stimulated muscle cells has a positive effect on neurons. However, the results could unfortunately not be reproduced in another independent experiment. The observed discrepancy in results could be affected due to the poor quality of the muscle cells being stimulated, and/or the poor quality of the neuronal cultures used for treatment (Figure 15D and 15E), which need to be thoroughly examined in future experiments.

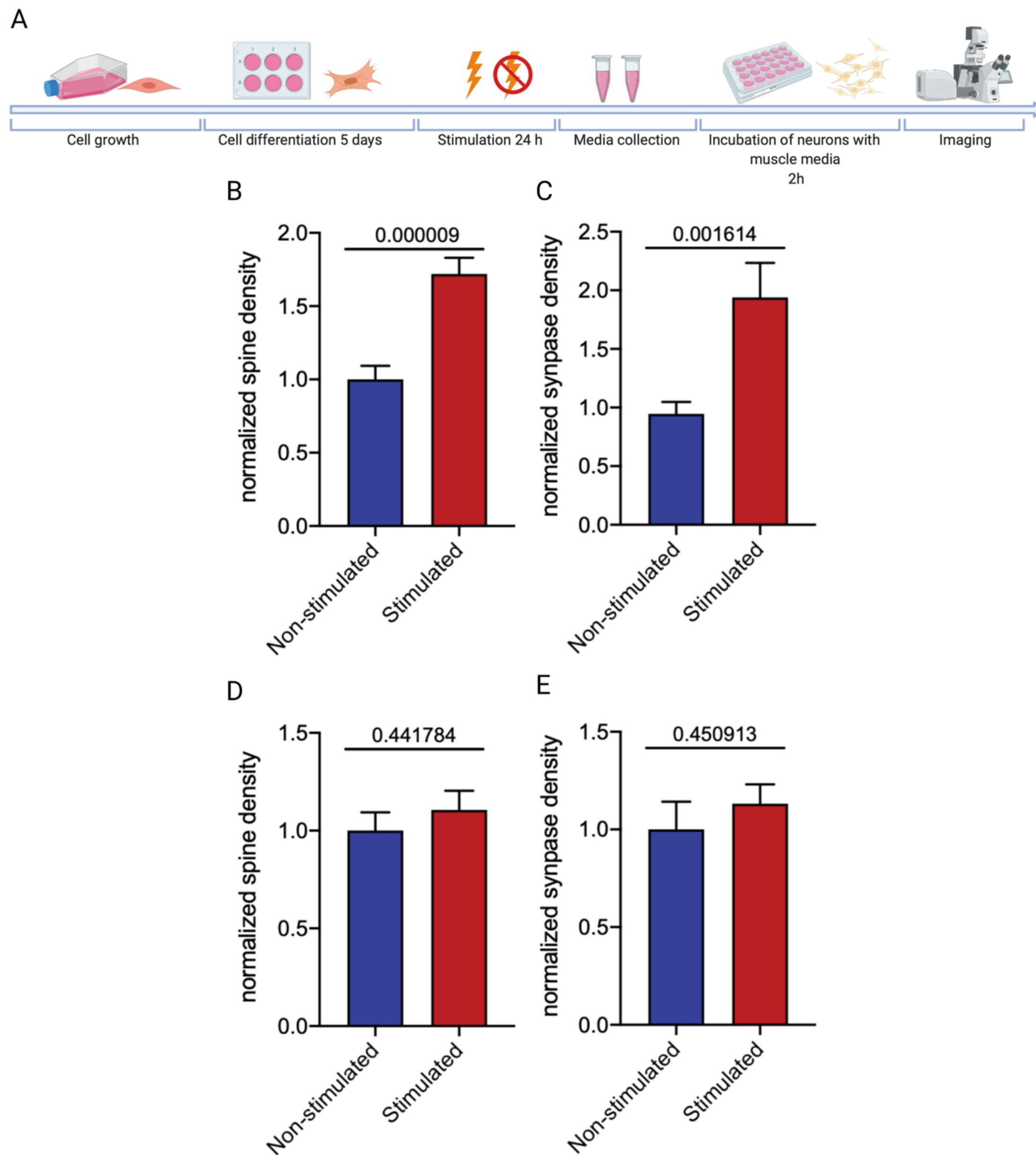

Figure 15: Incubation of neurons with muscle media and neuronal morphology. (A) Experimental design. Incubation of primary hippocampal neurons with the media collected from stimulated and non-stimulated muscle cell cultures. At DIV 7 70 % of neuronal maintenance media was removed and 30 % of media from muscle cells were added. Neurons were incubated for 2 h and then the mixed media was completely changed to 1 mL of maintenance media. At DIV 10 the neurons were fixed, and immunostaining was performed. (B) Spine density of neurons incubated with media from stimulated muscle cells relative to non-stimulated control (n = 3/3). Y-axis shows number of total spines divided per length of a chosen dendritic segment. (C) Synapse density of neurons incubated with media from stimulated muscle cells relative to non-stimulated control (n = 3/3). Y-axis shows synaptic density as percentage of colocalized puncta per image normalized to control. (D) Repetition of spine density experiment with stimulated and non-stimulated media (n = 6/6). (E) Repetition of synapse density experiment with stimulated and non-stimulated media (n = 6/6). Data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

In addition to previous experiments with inhibitors, we performed similar experiments where we overexpressed the expression of micro-RNA-409-5p and micro-RNA-501-3p using corresponding mimics packaged into LNPs. To confirm if the microRNA mimic treatment would lead to increased microRNA expression, first we tested the expression levels of both microRNAs in neurons after applying individual mimics and compared its expression to those from scramble control treated cells. When tested after 48 hours of treatment using qPCR, the analyses revealed that both microRNA mimics could indeed overexpress corresponding microRNAs compared to controls (Figure 16A and 16B). Next, the effect of overexpression of the two microRNAs on neuronal spine density and functional synapses were evaluated and the experiment was designed and performed as it was described above for anti-miRs. Analyses of the spine morphology revealed that overexpression of microRNA-409-5p led to increased spine density significantly (Figure 16C) compared to those from scramble control treated neurons. However, no significant difference in spine density was observed between microRNA-501-3p overexpressed and scramble control treated neurons, the trend was similar (Figure 16D). Next, we investigated the effect of overexpression of these two microRNAs on functional synapses. Unlike spine density, no significant difference in functional synapse number was observed between microRNA-409-5p overexpressed and scramble control treated neurons. A similar effect was also observed for healthy neurons overexpressing microRNA-501-3p.

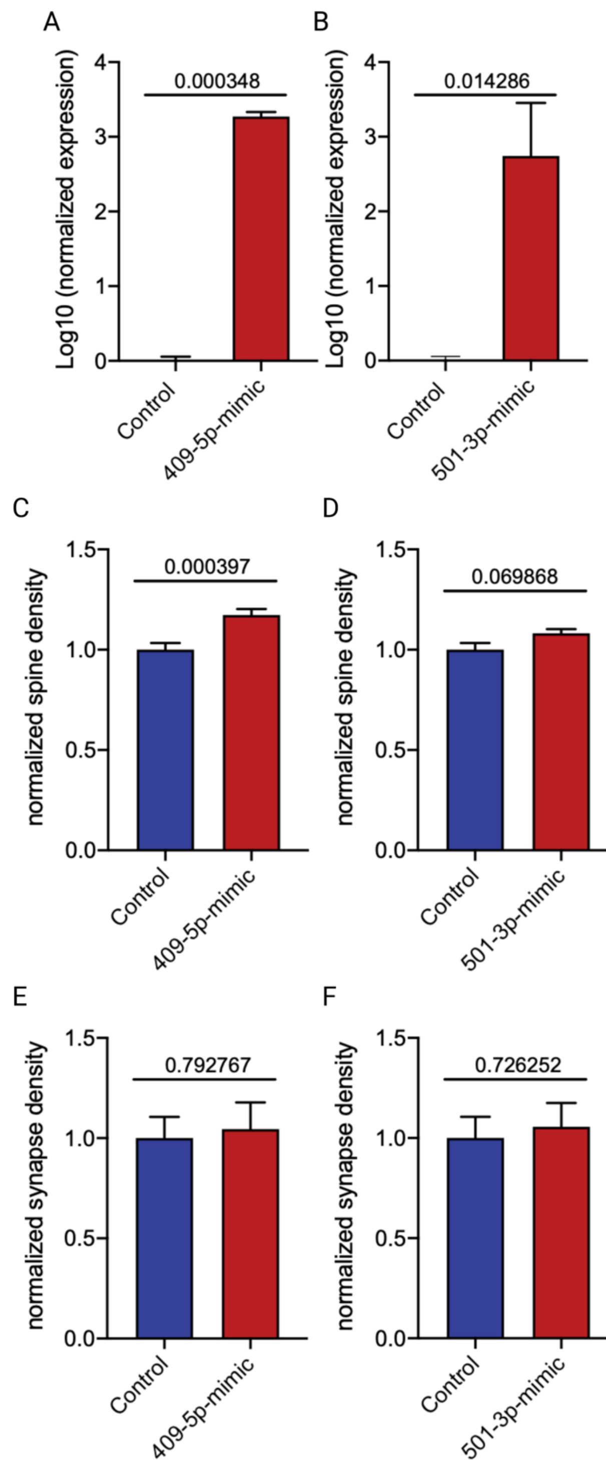

Figure 16: Overexpression of microRNAs and neuronal morphology. Quantification of (A) microRNA-409-5p and (B) microRNA-501-3p normalized expression levels by qPCR compared to control. Log transformation of the values was performed. Spine density in (C) microRNA-409-5p and in (D) microRNA-501-3p overexpressed neurons (n = 6/6) in comparison to control (n = 6). Y-axis shows number of total spines divided per length of a chosen dendritic segment. (E) microRNA-409-5p and (F) microRNA-501-3p overexpressed neurons (n = 6/6) in comparison to control (n = 6). Y-axis shows synaptic density as percentage of colocalized puncta per image normalized to control. Data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant.

Next, we tested the effect of over-expression of microRNA-409-5p and microRNA-501-3p on neuronal activity of healthy neurons. The experiment was performed as previously described for anti-miRs. For both microRNAs, we failed to see any significant difference in weighted mean firing rate (Figure 17A), number of bursts (Figure 17B) and number of network bursts (Figure 17C) when compared to those from scramble control treated neurons. These results suggest that overexpression of microRNA-409-5p and microRNA-501-3p in healthy neurons have limited impact on neuronal plasticity.

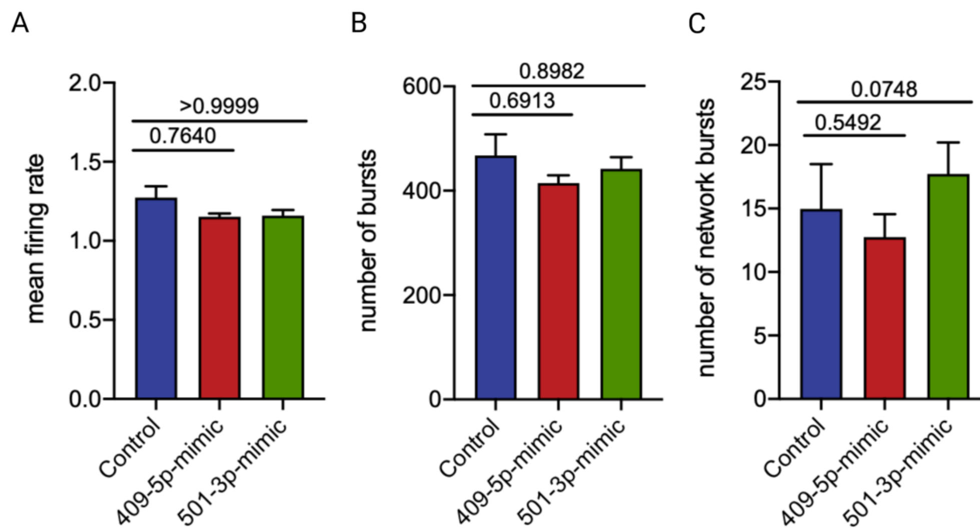

Figure 17: Neuronal electrical activity after microRNA overexpression. (D) Mean firing rate after treatment with anti-miR-409-5p and anti-miR-501-3p. (B) Number of bursts after treatment with anti-miR-409-5p and anti-miR-501-3p. (C) Number of spontaneous network-wide synchronized bursts after treatment with anti-miR-409-5p and anti-miR-501-3p. Data are presented as mean ± SEM for each time point. Kruskal-Wallis test with multiple comparison was performed, $p < 0.05$ was considered significant.

4 Discussion

Regular physical activity is one of the best predictors of lower mortality, decreased cancer rates, cerebrovascular diseases and improved brain function (Bernstein et al. 1994; Chudyk and Petrella 2011; Lear et al. 2017; Meng et al. 2020). Despite the ease and accessibility of such intervention many elderly people are not able to perform regular training. Understanding the molecular mechanisms behind benefits from exercise may help prevent cognitive decline in senior patients.

We first settled the major changes happening in response to exercise. The easiest approach to investigate this biological response was to investigate changes in the blood circulation system. Among many substances being investigated in blood, microRNAs have been attracting more and more attention in clinical research. They are believed to be involved in many diverse biological processes and regulate a significant part of coding protein genes (MacFarlane and R. Murphy 2010; Hammond 2015). Moreover, their identification in blood is proven to be robust due to their transportation within protein complexes or exosomes and thus low degradation and high stability in liquids (MacFarlane and R. Murphy 2010; Vickers et al. 2011).

To evaluate cognitive functions before and after exercise participants first underwent computer-based cognitive testing which included the Trail Making Test A and B, Wisconsin Card Sorting Test and Verbal Learning Memory Test. These tests are widely used in neurophysiological assessment. Despite the extreme simplicity of these tests, many parameters characterizing cognitive stage can be evaluated. Among them are cognitive flexibility, working memory, attention and executive function (Salthouse 2011). We confirmed the fact that physical training improves cognitive performance in individuals with no medical history of mental illness or cognitive impairment, which corresponds to studies published before (Mandolesi et al. 2018).

Our exploratory study identified that aerobic training caused upregulation of several circulating microRNAs in healthy volunteers. Two independent analyses of microRNA expression were performed: evaluation of eigen-expression and differential expression analysis. MicroRNAs that were part of a significantly upregulated cluster were also found to be highly expressed in a differential expression analysis. These microRNAs can be involved in multiple pathways and processes so we could not be sure if they are actually involved in neuronal function. For that reason, we first analyzed the expression pattern of the three most

deregulated microRNAs, namely microRNA-409-5p, microRNA-501-3p and microRNA-1271-5p, in mouse primary neuronal cultures. Although all three of them were highly expressed in human blood, only microRNA-409-5p and microRNA-501-3p were found to be expressed in mouse brain cultures by qPCR. We also analyzed existing literature on microRNA deregulation after exercise in humans and microRNA deregulation in cognitive impairment or experimental model with cognitive deficit. The idea was to find an overlap in microRNA signature between these two types of studies and then compare it to our own data. We noticed that microRNA-409-5p and microRNA-3p were also mentioned several times in other studies investigating mechanisms of cognitive decline. For example, microRNA-409-5p was found to be significantly downregulated in the whole brain of the mouse model of Alzheimer's disease. This result was then confirmed by qPCR and compared with wild type aged mice. MicroRNA-409-5p expression was much lower in the disease model at six and nine months old when compared to healthy mice (Luo et al. 2014). In another experimental model of isoflurane cognitive impairment microRNA array was performed on rat hippocampal tissues. MicroRNA-409-5p was also found to be downregulated in brain tissues of animals with cognitive decline in comparison to control (Luo et al. 2015). These two studies concluded that microRNA-409-5p may play an important role in the maintenance of normal neuronal functioning, that is why with the decrease expression of this microRNA cognitive disfunction follows. In contrast, other researchers explained downregulation of microRNA-409-5p in a mouse model of Alzheimer's disease as an adaptive protective mechanism in response to distortion of brain function induced by amyloid pathology. To prove the concept, they overexpressed this microRNA and observed neurotoxic reactions based on cell viability assay and morphology data. They however failed to show rescue effect of inhibition of microRNA-409-5p (Guo et al. 2019). As for microRNA-501-3p, it was found to be downregulated in the serum of patients with Alzheimer's disease. Not only was it less expressed in these patients, but its levels were also positively correlated with cognitive score. Interestingly, its expression pattern in postmortem temporal cortex tissue from the same patients was inversely higher compared to nondemented controls, so they assumed microRNA-501-3p plays rather a negative role in the development of cognitive impairment (Hara et al. 2017). In the contrary to this, another study on the mouse model of vascular dementia showed that microRNA-501-3p was upregulated in white matter after initiating disease condition. After inhibiting this microRNA systemically in mice they observed improved blood brain barrier, higher cerebral flow and better working memory (Toyama et al. 2018). While comparing our microRNAs with the studies done in sport research, we saw one paper where microRNA-501-3p was found to be

upregulated in blood after finishing a marathon (Fernandes-Sanjurjo et al. 2020). This information allowed us to further investigate these two microRNAs in the context of epigenetic regulation of brain function after physical activity.

After choosing our candidates we decided to first test the expression levels of these microRNAs in experimental exercise models. We first cultured immortalized mouse muscle cells and imitated exercise with electrical stimulation of these cells. We then checked how expression changes in muscle cells in response to stimulation. Indeed, one can see an upregulation trend in cells that were exposed to electrical simulation when compared to non-stimulated controls. We next decided to check if our microRNAs are expressed in mouse brains and if there are any changes in response to physical exercise. For that, we dissected hippocampi as the area that is widely investigated in studies of cognition and memory and analyzed expression after 18 weeks of voluntary wheel running compared to non-running control mice. Increased expression of microRNA-409-5p and microRNA-501-3p after exercise could be detected. The mechanism of how exercise effects circulating microRNAs however remains unknown and where exactly they come from is also not clear. Several suggestions have been made. It has been proposed that skeletal muscles produce and release microRNAs into the circulating system and then these microRNAs are being transported within the body (Aoi et al. 2013; Mayr et al. 2013). The other way around, microRNAs can be selectively taken by muscle cells from blood (Vickers et al. 2011). We cannot answer the question where these microRNAs come from, however we can state they can be identified in mouse muscle cells and mouse brain.

We hypothesized that these two microRNAs are responsible for normal neuronal functioning. To further test this hypothesis, we planned an experiment where we would inhibit the microRNAs and see what changes happen in neurons. As we already knew that our microRNAs of interest are expressed in mouse brains and their expression pattern changes in response to psychical loading, we decided to culture wild type primary mouse neurons and perform inhibition on them. For that we applied microRNA inhibitors in low concentrations on growing neurons. Successful inhibition could be seen with qPCR. Neurons were collected and RNA sequencing was performed in order to identify transcriptome changes after inhibiting the microRNAs. Interestingly, more than 2000 genes were upregulated under inhibition of both microRNA-409-5p and microRNA-501-3p. For a better understanding of the function and to give biological interpretation of these upregulated genes we performed gene ontology analysis. It showed that upregulated genes are involved in processes linked to neuronal apoptosis and autophagy, and that they suppress

such processes as axon growth and synapse maturation. In order to validate this finding, we set up two experiments to check neuronal morphology as cultured primary neurons are very sensitive to different manipulations and easy to image with a high-resolution STED microscope and analyze.

Spines are defined as small dendritic protrusions that are believed to serve as a region for future potential connection and synapse formation. They have thus been of interest for many studies as spine density is presumably correlated with memory and cognitive functions (Holtmaat and Svoboda 2009; Bosch and Hayashi 2012). In our study, we chose DiI dye staining to visualize individual spines on fixed primary neurons. Quantification of spine number revealed that neurons treated with microRNA-409-5p and microRNA-501-3p had much fewer spines when compared to scramble microRNA as control. We concluded that inhibition of these microRNAs indeed alters neuronal morphology on spine level, which goes in line with the gene ontology analysis.

Higher spine density does not necessarily mean that more functionally mature synapses will appear on the place of these spines. To investigate the number of formed synapses we performed another experiment where we stained primary neurons for the marker of presynaptic density Synaptophysin 1 and marker of postsynaptic density PSD-95. Colocalization of these two markers signifies presence of a formed mature synapse. Inhibition of our selected microRNAs also led to a decrease in the number of synapses when compared to the control. Based on the results from two independent imaging experiments we concluded that inhibition of microRNA-409-5p and microRNA-501-3p indeed negatively effects neuronal morphology and thus may play an important role in the maintenance of normal neuronal function in physiological conditions.

To better understand the effects on neuronal plasticity we needed not only to visualize changes in neuronal morphology but also to detect any potential changes in neuronal activity. One of the possibilities was to check for spontaneous mean neuronal firing rate as a marker of general neuronal activity, the number of bursts and synchronized network neuronal firing as an analogue of integrated firing in the brain that is necessary for memory formation. It is believed that more time precise integration of neuronal activity can lead to improved cognitive functions (Jutras and Buffalo 2010). We chose multi-electrode array as an electrophysiological instrument for the detection of spontaneous activity within neuronal cultures. This setup allows simultaneous and parallel detection of action potentials for each single well and electrode in it (Negri et al. 2020). We treated cells with microRNA inhibitors

in a similar way as described above for imaging experiments with the only difference that we mixed both inhibitors and applied them together this time. We suggested that inhibiting these microRNAs will also negatively alter neuronal activity. Indeed, after applying a cocktail of mixed inhibitors we could see a drastic decrease in neuron firing, bursts and synchronized network bursts.

As we strongly believed that these microRNAs are important for neuronal functioning and may indeed be relevant for cognition, we decided to check their expression in postmortem brain tissue from individuals with a known medical history of cognitive decline. By the time of this research, we already possessed samples of temporal cortex from patients with genetic frontotemporal dementia classified based on the three most known and often mutation C9, GRN or MAPT (Greaves and Rohrer 2019) and corresponding control tissue, as well as samples of the frontal cortex from patients with Alzheimer's disease at different Braak stages (Braak et al. 2006). We hypothesized that if these microRNAs are involved in neurocognitive functioning, their expression should be lower in case of impaired cognition. And we indeed saw changes for microRNA-409-5p: its expression was much lower in frontotemporal dementia and a similar trend could also be seen in later stages of Alzheimer's disease, although there was no significant difference. With an increased number of samples, we can however speculate that the difference could be significant. As for microRNA-501-3p, difference in its expression could not be identified. This phenomenon could be explained by differences in anatomical distribution, but we did not have an opportunity to investigate expression patterns in different brain regions.

Our next step was to overexpress the selected microRNA candidates and see if this can show a reversed effect on neuronal plasticity. We fist decided to simulate a more natural and less selective way of overexpressing our exercise-induced microRNAs. We suggested that as we observed upregulation of microRNAs on muscle cells after electrical stimulation, treating neurons with media from these cells would imitate the effect of microRNA overexpressing. For that we cultured and stimulated immortalized mouse muscle cells and collected the media from stimulated and control groups. We then cultured mouse primary neurons and treated them not with microRNA this time, but with the media we obtained in the previous step. We then performed imaging experiments as we did for the inhibition experiment. In pilot experiment we saw a drastic effect of stimulated media on spine and synapse density. This is in line with a paper published recently where the authors transferred plasma from running mice into aged mice and this led to reversed age-related cognitive decline, improved learning and increased neurogenesis (Horowitz et al. 2020). However, during the repetition of the

experiment that was started in order to increase the number of replicates, we did not observe this phenomenon anymore. What is nonetheless interesting is that after revising the fixed and stained coverslip together with an experienced scientist, we noticed that neurons for the pilot experiment visually looked unhealthier than the ones from later interventions. This could mean that the effect of so-called exercised media might only be seen when neurons are already functionally impaired.

We then performed overexpression experiments on mouse primary hippocampal cultures with microRNA mimics, similar to what was done with the inhibitors. The magnitude of overexpression effect was much stronger when compared to inhibitors, although both mimic and inhibitor were ten times diluted. We saw an increased spine number after incubation with mimics. Nevertheless, we did not observe any effect of microRNA-409-5p or microRNA-501-3p mimic on mature synapse number. No changes were also observed for electrical activity. This can be explained by the necessity to challenge neurons in order to see a positive effect of microRNA mimics. In the end, we did not set a goal of changing an already healthy system.

5 Summary

Epigenetics has great potential to provide us with answers on how benefits from physical exercise can be applied to cognitively impaired elderly patients and reverse age-related and disease-associated loss of normal cognitive function. MicroRNA is a novel tool to manipulate a wide range of physiological processes as single microRNA regulates the functions of multiple genes.

We found two microRNAs that were upregulated in human blood as well as in stimulated mouse muscle cells and mouse brain after physical exercise, and we validated their role in neuronal plasticity. Inhibition of microRNA-409-5p and microRNA-501-3p negatively alters neuronal morphology and electrophysiological activity in mouse wild type hippocampal neurons. Moreover, expression of microRNA-409-5p was found to be decreased in patients with genetic frontotemporal dementia. Transplantation of media from electrically stimulated muscle may alter neuronal morphology. Overexpression of microRNA-409-5p and microRNA-501-3p increases spine number but does not have an effect on mature synapse number or action potentials of wild type healthy hippocampal neurons.

Further studies are needed to validate experimentally other microRNAs from the identified cluster. More behavior experiments have to be conducted in order to functionally prove the role of these microRNAs in memory and cognition. In the future, cumulative scientific knowledge can lead to the discovery of several microRNAs, the combination of which can be systemically used in clinical practice as an efficient treatment to reverse cognitive decline.

6 Appendix

We generally believed that all microRNAs that are part of the module midnightblue should be investigated in terms of their effect on neuronal functions. For that reason, we additionally took another microRNA from the highly deregulated module described above. MicroRNA-100-5p was suggested as cerebrospinal fluid marker and was found to be upregulated with Alzheimer's disease (Denk et al. 2015). This microRNA was also found to be decreased in brain samples of mice models of Alzheimer's disease at early stages and increased at later stages (Ye et al. 2015). Due to mentioned studies and the presence of microRNA-100-5p in our cluster we performed several additional functional experiments using LNPs packaged with inhibitor of microRNA-100-5p (anti-miR-100-5p). First, the inhibition itself was checked in mouse primary neuronal cultures. As in other experiments, neurons incubated with anti-miR-100-5p showed a strong reduction in expression (Figure S1A). Next, we checked neuronal morphology after anti-miR-100-5p application. Both spine density and synapse density were reduced (Figure S1B and S1C). Finally, we performed recording of neuronal activity. A strong reduction in all tested parameters was observed after incubation with anti-miR-100-5p: mean firing rate, number of bursts and number of network bursts (Figure S1D, S1E and S1F). Other experiments similar to those described for two other microRNAs were not performed due to time limitations.

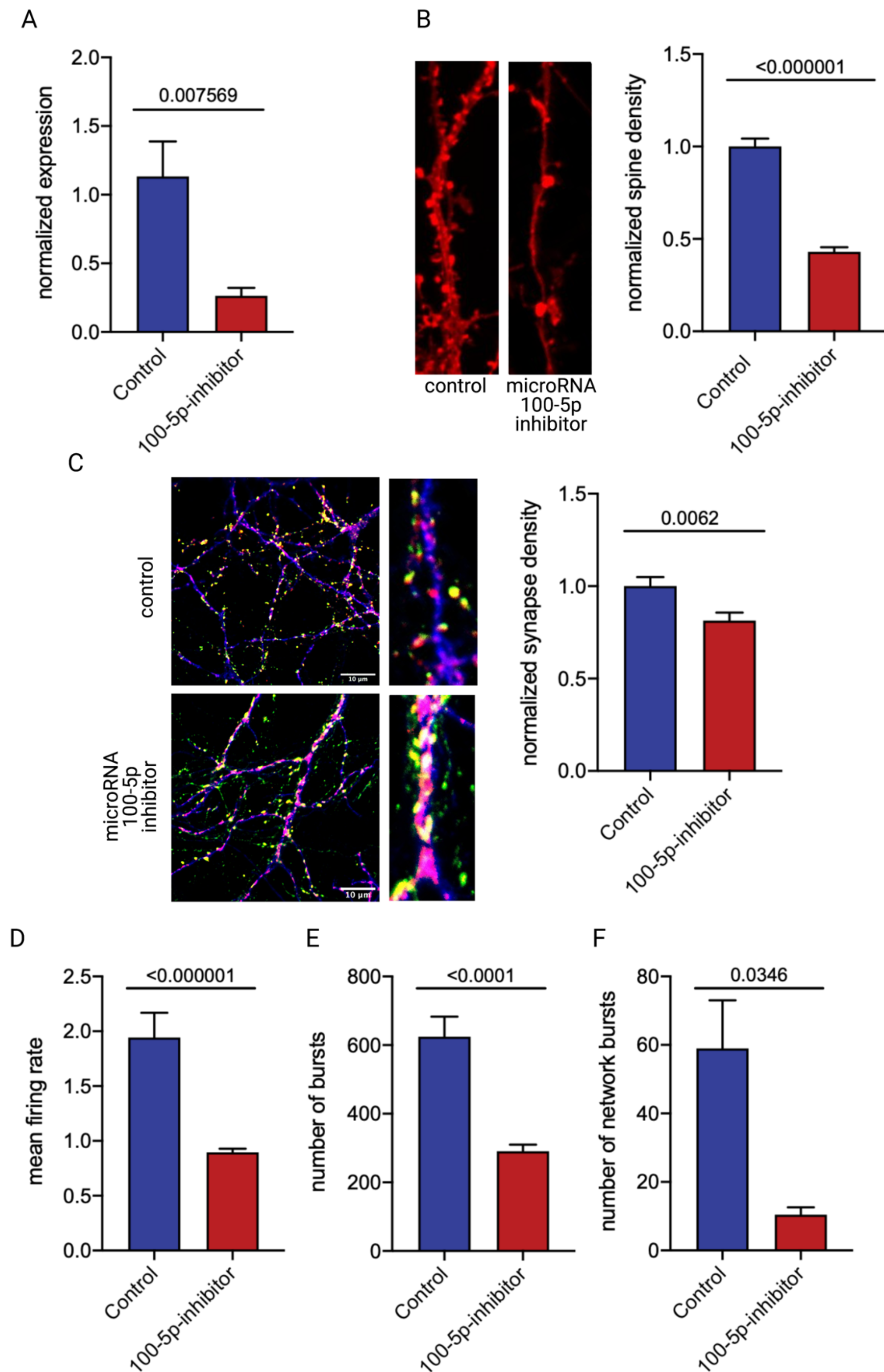

Figure S1: Neuronal morphology and electrical activity after microRNA-100-5p inhibition. (A) Quantification of microRNA-100-5p expression by qPCR after applying anti-miR-100-5p. Bar charts show normalized expression relative to control, data shown as means ± SEM, unpaired nonparametric t-test was used, $p < 0.05$ was considered significant. (B) Spine density analysis with example images of DiI staining of control and inhibited samples. Y-axis shows number of total spines divided per length of a chosen dendritic segment, data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant. (n = 6/6). (C) Mature synapse density analysis with example overview and zoomed imaged of

control and inhibited samples. Colocalized signals from PSD-95 and Synaptophysin 1 are shown in yellow. Y-axis shows synapse density as percentage of colocalized puncta per image normalized to control, data are presented as mean ± SEM, unpaired parametric t-test was used, $p < 0.05$ was considered significant. (D) Mean firing rate after treatment with anti-miR-100-5p. (B) Number of bursts after treatment with anti-miR-100-5p. (C) Number of spontaneous network-wide synchronized bursts after treatment with anti-miR-100-5p. Data are presented as mean ± SEM for each time point. Kruskal-Wallis test with multiple comparison was performed, $p < 0.05$ was considered significant.

7 References

Alberti C, Cochella L (2017): A framework for understanding the roles of miRNAs in animal development. Development 144, 2548–2559

Aoi W, Ichikawa H, Mune K, Tanimura Y, Mizushima K, Naito Y, Yoshikawa T (2013): Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. Front Physiol 4, 80

Au R, Seshadri S, Knox K, Beiser A, Himali JJ, Cabral HJ, Auerbach S, Green RC, Wolf PA, McKee AC (2012): The Framingham Brain Donation Program: neuropathology along the cognitive continuum. Curr Alzheimer Res 9, 673–686

Baggish AL, Hale A, Weiner RB, Lewis GD, Systrom D, Wang F, Wang TJ, Chan SY (2011): Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training: Circulating microRNA in exercise. J Physiol 589, 3983–3994

Banzet S, Chennaoui M, Girard O, Racinais S, Drogou C, Chalabi H, Koulmann N (2013): Changes in circulating microRNAs levels with exercise modality. J Appl Physiol 115, 1237–1244

Barbato C, Giorgi C, Catalanotto C, Cogoni C (2008): Thinking about RNA? MicroRNAs in the brain. Mamm Genome 19, 541–551

Barber JL, Zellars KN, Barringhaus KG, Bouchard C, Spinale FG, Sarzynski MA (2019): The Effects of Regular Exercise on Circulating Cardiovascular-related MicroRNAs. Sci Rep 9, 7527

Bartel DP (2004): MicroRNAs. Cell 116, 281–297

Baune BT, Wiede F, Braun A, Golledge J, Arolt V, Koerner H (2008): Cognitive dysfunction in mice deficient for TNF- and its receptors. Am J Med Genet B Neuropsychiatr Genet 147B, 1056–1064

Bayraktar R, Van Roosbroeck K, Calin GA (2017): Cell-to-cell communication: microRNAs as hormones. Mol Oncol 11, 1673–1686

Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ (2003): Dicer is essential for mouse development. Nat Genet 35, 215–217

Bernstein L, Henderson BE, Hanisch R, Sullivan-Halley J, Ross RK (1994): Physical exercise and reduced risk of breast cancer in young women. J Natl Cancer Inst 86, 1403–1408

Bosch M, Hayashi Y (2012): Structural plasticity of dendritic spines. Curr Opin Neurobiol 22, 383–388

Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K (2006): Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 112, 389–404

Bye A, Røsjø H, Aspenes ST, Condorelli G, Omland T, Wisløff U (2013): Circulating MicroRNAs and Aerobic Fitness – The HUNT-Study. PLoS ONE 8, e57496

Chong MMW, Zhang G, Cheloufi S, Neubert TA, Hannon GJ, Littman DR (2010): Canonical and alternate functions of the microRNA biogenesis machinery. Genes Dev 24, 1951–1960

Chudyk A, Petrella RJ (2011): Effects of exercise on cardiovascular risk factors in type 2 diabetes: a meta-analysis. Diabetes Care 34, 1228–1237

Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, Elavsky S, Marquez DX, Hu L, Kramer AF (2006): Aerobic exercise training increases brain volume in aging humans. J Gerontol A Biol Sci Med Sci 61, 1166–1170

Cui SF, Wang C, Yin X, Tian D, Lu QJ, Zhang CY, Chen X, Ma JZ (2016): Similar Responses of Circulating MicroRNAs to Acute High-Intensity Interval Exercise and Vigorous-Intensity Continuous Exercise. Front Physiol 7, 102

Da Silva ND, Fernandes T, Soci UPR, Monteiro AWA, Phillips MI, De Oliveira EM (2012): Swimming Training in Rats Increases Cardiac MicroRNA-126 Expression and Angiogenesis. Med Sci Sports Exerc 44, 1453–1462

Danese E, Benati M, Sanchis-Gomar F, Tarperi C, Salvagno GL, Paviati E, Montagnana M, Schena F, Lippi G (2018): Influence of middle-distance running on muscular micro RNAs. Scand J Clin Lab Invest 78, 165–170

Davidsen PK, Gallagher IJ, Hartman JW, Tarnopolsky MA, Dela F, Helge JW, Timmons JA, Phillips SM (2011): High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. J Appl Physiol 110, 309–317

De la Rosa A, Solana E, Corpas R, Bartrés-Faz D, Pallàs M, Vina J, Sanfeliu C, Gomez-Cabrera MC (2019): Long-term exercise training improves memory in middle-aged men and modulates peripheral levels of BDNF and Cathepsin B. Sci Rep 9, 3337

Denham J, Prestes PR (2016): Muscle-Enriched MicroRNAs Isolated from Whole Blood Are Regulated by Exercise and Are Potential Biomarkers of Cardiorespiratory Fitness. Front Genet 7, 196

Denk J, Boelmans K, Siegismund C, Lassner D, Arlt S, Jahn H (2015): MicroRNA Profiling of CSF Reveals Potential Biomarkers to Detect Alzheimer`s Disease. PLoS One 10, e0126423

Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ (2004): Processing of primary microRNAs by the Microprocessor complex. Nature 432, 231–235

DESGESCO (Dementia Genetics Spanish Consortium), EADB (Alzheimer Disease European DNA biobank), IFGC (International FTD-Genomics Consortium), IPDGC (The International Parkinson Disease Genomics Consortium), RiMod-FTD (Risk and Modifying factors in Fronto-Temporal Dementia), Netherlands Brain Bank (NBB), The GIFT (Genetic Investigation in Frontotemporal Dementia and Alzheimer's Disease) Study Group, van der Lee SJ, Conway OJ, Jansen I, et al. (2020): Correction to: A nonsynonymous mutation in PLCG2 reduces the risk of Alzheimer's disease, dementia with Lewy bodies and frontotemporal dementia, and increases the likelihood of longevity. Acta Neuropathol 139, 959–962

DGPPN, DGN (2016): Demenzen. S3-Leitlinie der Deutschen Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde (DGPPN) and Deutschen Gesellschaft für Neurologie (DGN). https://www.awmf.org/leitlinien/detail/ll/038-013.html; accessed on 24.01.2016

Dias RG, Silva MSM, Duarte NE, Bolani W, Alves CR, Junior JRL, da Silva JL, de Oliveira PA, Alves GB, de Oliveira EM, et al. (2015): PBMCs express a transcriptome signature predictor of oxygen uptake responsiveness to endurance exercise training in men. Physiol Genomics 47, 13–23

Do K, Laing BT, Landry T, Bunner W, Mersaud N, Matsubara T, Li P, Yuan Y, Lu Q, Huang H (2018): The effects of exercise on hypothalamic neurodegeneration of Alzheimer's disease mouse model. PLoS ONE 13, e0190205

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013): STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21

Dogini DB, Ribeiro PAO, Rocha C, Pereira TC, Lopes-Cendes I (2008): MicroRNA expression profile in murine central nervous system development. J Mol Neurosci 35, 331–337

Domańska-Senderowska D, Laguette M-J, Jegier A, Cięszczyk P, September A, Brzeziańska-Lasota E (2019): MicroRNA Profile and Adaptive Response to Exercise Training: A Review. Int J Sports Med 40, 227–235

Dong H, Li J, Huang L, Chen X, Li D, Wang T, Hu C, Xu J, Zhang C, Zen K, et al. (2015): Serum MicroRNA Profiles Serve as Novel Biomarkers for the Diagnosis of Alzheimer's Disease. Dis Markers 2015, 1–11

Erickson KI, Prakash RS, Voss MW, Chaddock L, Hu L, Morris KS, White SM, Wójcicki TR, McAuley E, Kramer AF (2009): Aerobic fitness is associated with hippocampal volume in elderly humans. Hippocampus 19, 1030–1039

Esteller M (2011): Non-coding RNAs in human disease. Nat Rev Genet 12, 861–874

Fabel Klaus, Fabel Konstanze, Tam B, Kaufer D, Baiker A, Simmons N, Kuo CJ, Palmer TD (2003): VEGF is necessary for exercise-induced adult hippocampal neurogenesis. Eur J Neurosci 18, 2803–2812

Fenn AM, Smith KM, Lovett-Racke AE, Guerau-de-Arellano M, Whitacre CC, Godbout JP (2013): Increased micro-RNA 29b in the aged brain correlates with the reduction of insulin-like growth factor-1 and fractalkine ligand. Neurobiol Aging 34, 2748–2758

Fernandes J, Vieira AS, Kramer-Soares JC, Da Silva EA, Lee KS, Lopes-Cendes I, Arida RM (2018): Hippocampal microRNA-mRNA regulatory network is affected by physical exercise. Biochim Biophys Acta Gen Subj 1862, 1711–1720

Fernandes-Sanjurjo M, Ubeda N, Fernandes-Garcia B, Dell Valle M, Lee KS, Ramires de Molina A, Crespo MC, Roberto MH, Patricia CA et al. (2020): Exercise dose affects the circulating microRNA profile in response to acute endurance exercise in male amateur runners. Scand J Med Sci Sports 30, 1896–1907

Fischer A (2016): Environmental enrichment as a method to improve cognitive function. What can we learn from animal models? Neuroimage 131, 42–47

Friedländer MR, Chen W, Adamidi C, Maaskola J, Einspanier R, Knespel S, Rajewsky N (2008): Discovering microRNAs from deep sequencing data using miRDeep. Nat Biotechnol 26, 407–415

Friedman RC, Farh KKH, Burge CB, Bartel DP (2008): Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19, 92–105

Gebert LFR, MacRae IJ (2019): Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 20, 21–37

Grabrucker A, Vaida B, Bockmann J, Boeckers TM (2009): Synaptogenesis of hippocampal neurons in primary cell culture. Cell Tissue Res 338, 333–341

Greaves CV, Rohrer JD (2019): An update on genetic frontotemporal dementia. J Neurol 266, 2075–2086

Guescini M, Canonico B, Lucertini F, Maggio S, Annibalini G, Barbieri E, Luchetti F, Papa S, Stocchi V (2015): Muscle Releases Alpha-Sarcoglycan Positive Extracellular Vesicles Carrying miRNAs in the Bloodstream. PLoS ONE 10, e0125094

Guo H, Ingolia NT, Weissman JS, Bartel DP (2010): Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466, 835–840

Guo J, Cai Y, Ye X, Ma N, Wang Y, Yu B, Wan J (2019): MiR-409-5p as a Regulator of Neurite Growth Is Down Regulated in APP/PS1 Murine Model of Alzheimer's Disease. Front Neurosci 13, 1264

Ha M, Kim VN (2014): Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15, 509–524

Hammond SM (2015): An overview of microRNAs. Adv Drug Deliv Rev 87, 3–14

Hara N, Kikuchi M, Miyashita A, Hatsuta H, Saito Y, Kasuga K, Murayama S, Ikeuchi T, Kuwano R (2017): Serum microRNA miR-501-3p as a potential biomarker related to the progression of Alzheimer's disease. Acta Neuropathol Commun 5, 10

Hebert SS, Horre K, Nicolai L, Papadopoulou AS, Mandemakers W, Silahtaroglu AN, Kauppinen S, Delacourte A, De Strooper B (2008): Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. Proc Natl Acad Sci USA 105, 6415–6420

Holtmaat A, Svoboda K (2009): Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci 10, 647–658

Horowitz AM, Fan X, Bieri G, Smith LK, Sanchez-Diaz CI, Schroer AB, Gontier G, Casaletto KB, Kramer JH, Williams KE, Villeda SA (2020): Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain. Science 369, 167–173

Hu Z, Zhao J, Hu T, Luo Y, Zhu J, Li Z (2015): miR-501-3p mediates the activity-dependent regulation of the expression of AMPA receptor subunit GluA1. J Cell Biol 208, 949–959

Huang W: MicroRNAs: Biomarkers, Diagnostics, and Therapeutics. In: Huang J, Borchert GM, Dou D, Huan J, Lan W, Tan M, Wu B (Eds.): Bioinformatics in MicroRNA Research (Methods in Molecular Biology). Band 1617; Springer New York, New York 2017, 57–67

Islam MR, Lbik D, Sakib MS, Maximilian Hofmann R, Berulava T, Jiménez Mausbach M, Cha J, Goldberg M, Vakhtang E, Schiffmann C, et al. (2021): Epigenetic gene expression links heart failure to memory impairment. EMBO Mol Med 13

Jonas S, Izaurralde E (2015): Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 16, 421–433

Jutras MJ, Buffalo EA (2010): Synchronous neural activity and memory formation. Curr Opin Neurobiol 20, 150–155

Knopman DS, Petersen RC (2014): Mild Cognitive Impairment and Mild Dementia: A Clinical Perspective. Mayo Clin Proc 89, 1452–1459

Kongs SK, Thompson LL, Grant IL, Heaton RK (Hrsg.): Wisconsin card sorting test-64 card version (WCST-64). Professional Manual; Psychological Assessment Resources Inc, 2000

Kumar P, Dezso Z, MacKenzie C, Oestreicher J, Agoulnik S, Byrne M, Bernier F, Yanagimachi M, Aoshima K, Oda Y (2013): Circulating miRNA Biomarkers for Alzheimer's Disease. PLoS ONE 8, e69807

Lear SA, Hu W, Rangarajan S, Gasevic D, Leong D, Iqbal R, Casanova A, Swaminathan S, Anjana RM, Kumar R, et al. (2017): The effect of physical activity on mortality and cardiovascular disease in 130 000 people from 17 high-income, middle-income, and low-income countries: the PURE study. Lancet 390, 2643–2654

Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim VN (2003): The nuclear RNase III Drosha initiates microRNA processing. Nature 425, 415–419

Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD (2012): The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 28, 882–883

Leidinger P, Backes C, Deutscher S, Schmitt K, Mueller SC, Frese K, Haas J, Ruprecht K, Paul F, Stähler C, et al. (2013): A blood based 12-miRNA signature of Alzheimer disease patients. Genome Biol 14, R78

Leiter O, Seidemann S, Overall RW, Ramasz B, Rund N, Schallenberg S, Grinenko T, Wielockx B, Kempermann G, Walker TL (2019): Exercise-Induced Activated Platelets Increase Adult Hippocampal Precursor Proliferation and Promote Neuronal Differentiation. Stem Cell Reports 12, 667–679

Lin PJ, Zhong Y, Fillit HM, Cohen JT, Neumann PJ (2017): Hospitalizations for ambulatory care sensitive conditions and unplanned readmissions among Medicare beneficiaries with Alzheimer's disease. Alzheimers Dement 13, 1174–1178

Liu C, Wang J, Li L, Xue L, Zhang Y, Wang P (2014): MicroRNA-135a and -200b, potential Biomarkers for Alzheimer′s disease, regulate β secretase and amyloid precursor protein. Brain Res 1583, 55–64

Liu CG, Song J, Zhang YQ, Wang PC (2014a): MicroRNA-193b is a regulator of amyloid precursor protein in the blood and cerebrospinal fluid derived exosomal microRNA-193b is a biomarker of Alzheimer's disease. Mol Med Rep 10, 2395–2400

Liu CG, Wang JL, Li L, Wang PC (2014b): MicroRNA-384 regulates both amyloid precursor protein and β-secretase expression and is a potential biomarker for Alzheimer's disease. Int J Mol Med 34, 160–166

Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, Xiao C, Bezzerides V, Boström P, Che L, et al. (2015): miR-222 Is Necessary for Exercise-Induced Cardiac Growth and Protects against Pathological Cardiac Remodeling. Cell Metab 21, 584–595

Livak KJ, Schmittgen TD (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408

Luo H, Wu Q, Ye X, Xiong Y, Zhu J, Xu J, Diao Y, Zhang D, Wang M, Qiu J, et al. (2014): Genome-Wide Analysis of miRNA Signature in the APPswe/PS1ΔE9 Mouse Model of Alzheimer's Disease. PLoS ONE 9, e101725

Luo T, Yin S, Shi R, Xu C, Wang Y, Cai J, Yue Y, Wu A (2015): miRNA Expression Profile and Involvement of Let-7d-APP in Aged Rats with Isoflurane-Induced Learning and Memory Impairment. PLoS ONE 10, e0119336

Maass A, Düzel S, Brigadski T, Goerke M, Becke A, Sobieray U, Neumann K, Lövdén M, Lindenberger U, Bäckman L, et al. (2016): Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. Neuroimage 131, 142–154

MacFarlane L-A, R. Murphy P (2010): MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics 11, 537–561

Malchow B, Keller K, Hasan A, Dörfler S, Schneider-Axmann T, Hillmer-Vogel U, Honer WG, Schulze TG, Niklas A, Wobrock T, et al. (2015): Effects of Endurance Training Combined With Cognitive Remediation on Everyday Functioning, Symptoms, and Cognition in Multiepisode Schizophrenia Patients. Schizophr Bull 41, 847–858

Malchow B, Keeser D, Keller K, Hasan A, Rauchmann B-S, Kimura H, Schneider-Axmann T, Dechent P, Gruber O, Ertl-Wagner B, et al. (2016): Effects of endurance training on brain structures in chronic schizophrenia patients and healthy controls. Schizophr Bull 173, 182–191

Mandolesi L, Polverino A, Montuori S, Foti F, Ferraioli G, Sorrentino P, Sorrentino G (2018): Effects of Physical Exercise on Cognitive Functioning and Wellbeing: Biological and Psychological Benefits. Front Psychol 9, 509

Margolis LM, Lessard SJ, Ezzyat Y, Fielding RA, Rivas DA (2016): Circulating MicroRNA Are Predictive of Aging and Acute Adaptive Response to Resistance Exercise in Men. J Gerontol A Biol Sci Med Sci 72, 1319-1326

Martinelli NC, Cohen CR, Santos KG, Castro MA, Biolo A, Frick L, Silvello D, Lopes A, Schneider S, Andrades ME, et al. (2014): An Analysis of the Global Expression of MicroRNAs in an Experimental Model of Physiological Left Ventricular Hypertrophy. PLoS ONE 9, e93271

Matranga C, Tomari Y, Shin C, Bartel DP, Zamore PD (2005): Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. Cell 123, 607–620

Mayr M, Zampetaki A, Kiechl S (2013): MicroRNA biomarkers for failing hearts? Eur Heart J 34, 2782–2783

McAfoose J, Baune BT (2009): Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev 33, 355–366

Mellios N, Huang H-S, Grigorenko A, Rogaev E, Akbarian S (2008): A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. Hum Mol Genet 17, 3030–3042

Meng Q, Lin M-S, Tzeng IS (2020): Relationship Between Exercise and Alzheimer's Disease: A Narrative Literature Review. Front Neurosci 14, 131

Müller H, Hasse-Sander I, Horn R, Helmstaedter C, Elger CE (1997): Rey Auditory-Verbal Learning Test: structure of a modified German version. J Clin Psychol 53, 663–671

Nagaraj S, Laskowska-Kaszub K, Dębski KJ, Wojsiat J, Dąbrowski M, Gabryelewicz T, Kuźnicki J, Wojda U (2017): Profile of 6 microRNA in blood plasma distinguish early stage Alzheime's disease patients from non-demented subjects. Oncotarget 8, 16122-16143

Negri J, Menon V, Young-Pearse TL (2020): Assessment of Spontaneous Neuronal Activity In Vitro Using Multi-Well Multi-Electrode Arrays: Implications for Assay Development. eNeuro 7

Nichols E, Szoeke CEI, Vollset SE, Abbasi N, Abd-Allah F, Abdela J, Aichour MTE, Akinyemi RO, Alahdab F, Asgedom SW, et al. (2019): Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18, 88–106

Nicholson AW (2014): Ribonuclease III mechanisms of double-stranded RNA cleavage. Wiley Interdiscip Rev RNA 5, 31–48

Nie Y, Sato Y, Wang C, Yue F, Kuang S, Gavin TP (2016): Impaired exercise tolerance, mitochondrial biogenesis, and muscle fiber maintenance in miR-133a-deficient mice. FASEB J 30, 3745–3758

Nielsen S, Scheele C, Yfanti C, Akerström T, Nielsen AR, Pedersen BK, Laye MJ, Laye M (2010): Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. J Physiol 588, 4029–4037

Nielsen S, Åkerström T, Rinnov A, Yfanti C, Scheele C, Pedersen BK, Laye MJ (2014): The miRNA Plasma Signature in Response to Acute Aerobic Exercise and Endurance Training. PLoS ONE 9, e87308

Ogasawara R, Akimoto T, Umeno T, Sawada S, Hamaoka T, Fujita S (2016): MicroRNA expression profiling in skeletal muscle reveals different regulatory patterns in high and low responders to resistance training. Physiol Genomics 48, 320–324

Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, Nakagawa A, Yoneda Y, Tsukihara T (2009): A high-resolution structure of the pre-microRNA nuclear export machinery. Science 326, 1275–1279

Parr EB, Camera DM, Burke LM, Phillips SM, Coffey VG, Hawley JA (2016): Circulating MicroRNA Responses between 'High' and 'Low' Responders to a 16-Wk Diet and Exercise Weight Loss Intervention. PLoS ONE 11, e0152545

Pauli A, Rinn JL, Schier AF (2011): Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet 12, 136–149

Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, Sloan R, Gage FH, Brown TR, Small SA (2007): An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci USA 104, 5638–5643

Pierson WR, Montoye HJ (1958): Movement time, reaction time, and age. J Gerontol 13, 418–421

Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, Burke JR, Hurd MD, Potter GG, Rodgers WL, et al. (2007): Prevalence of Dementia in the United States: The Aging, Demographics, and Memory Study. Neuroepidemiology 29, 125–132

Prabhakar P, Chandra SR, Christopher R (2017): Circulating microRNAs as potential biomarkers for the identification of vascular dementia due to cerebral small vessel disease. Age Ageing 46, 861–864

Prince M, Wimo A, Guerchet M, Ali GC, Wu YT, Prina M (Eds.): World Alzheimer Report 2015: the Global Impact of Dementia: An Analysis of Prevalence, Incidence, Cost and Trends. https://www.alzint.org/resource/world-alzheimer-report-2015/; accessed on 15.05.2021

Radom-Aizik S, Zaldivar F, Oliver S, Galassetti P, Cooper DM (2010): Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. J Appl Physiol 109, 252–261

Radom-Aizik S, Zaldivar F, Leu SY, Adams GR, Oliver S, Cooper DM (2012): Effects of Exercise on microRNA Expression in Young Males Peripheral Blood Mononuclear Cells. Clin Trans Sci 5, 32–38

Radom-Aizik S, Zaldivar F, Haddad F, Cooper DM (2013): Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults. J Appl Physiol 114, 628–636

Radom-Aizik S, Zaldivar FP, Haddad F, Cooper DM (2014): Impact of brief exercise on circulating monocyte gene and microRNA expression: Implications for atherosclerotic vascular disease. Brain Behav Immun 39, 121–129

Rani A, O'Shea A, Ianov L, Cohen RA, Woods AJ, Foster TC (2017): miRNA in Circulating Microvesicles as Biomarkers for Age-Related Cognitive Decline. Front Aging Neurosci 9, 323

Rauchmann BS, Ghaseminejad F, Keeser D, Keller-Varady K, Schneider-Axmann T, Takahashi S, Karali T, Helms G, Dechent P, Maurus I, et al. (2019): The impact of endurance training and table soccer on brain metabolites in schizophrenia. Brain Imaging Behav 14, 515-526

Reitan RM, Wolfson D: The Halstead-Reitan neuropsychological test battery: theory and clinical interpretation. Neuropsychology Press, Tucson, Arizona 1985

Roeh A, Malchow B, Levold K, Labusga M, Keller-Varady K, Schneider-Axmann T, Wobrock T, Schmitt A, Falkai P, Hasan A (2020): Effects of Three Months of Aerobic Endurance Training on Motor Cortical Excitability in Schizophrenia Patients and Healthy Subjects. Neuropsychobiology 79, 100–107

Russell AP, Lamon S, Boon H, Wada S, Güller I, Brown EL, Chibalin AV, Zierath JR, Snow RJ, Stepto N, et al. (2013): Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. J Physiol 591, 4637–4653

Safdar A, Abadi A, Akhtar M, Hettinga BP, Tarnopolsky MA (2009): miRNA in the Regulation of Skeletal Muscle Adaptation to Acute Endurance Exercise in C57Bl/6J Male Mice. PLoS ONE 4, e5610

Salthouse TA (2011): What cognitive abilities are involved in trail-making performance? Intelligence 39, 222–232

Sanders LMJ, Hortobágyi T, la Bastide-van Gemert S, van der Zee EA, van Heuvelen MJG (2019): Dose-response relationship between exercise and cognitive function in older adults with and without cognitive impairment: A systematic review and meta-analysis. PLoS ONE 14, e0210036

Satoh JI, Kino Y, Niida S (2015): MicroRNA-Seq Data Analysis Pipeline to Identify Blood Biomarkers for Alzheimer's Disease from Public Data. Biomark Insights 10, 21–31

Sawada S, Kon M, Wada S, Ushida T, Suzuki K, Akimoto T (2013): Profiling of Circulating MicroRNAs after a Bout of Acute Resistance Exercise in Humans. PLoS ONE 8, e70823

Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME (2006): A brain-specific microRNA regulates dendritic spine development. Nature 439, 283–289

Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V (2004): Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 5, R13

Shah R, Yeri A, Das A, Courtright-Lim A, Ziegler O, Gervino E, Ocel J, Quintero-Pinzon P, Wooster L, Bailey CS, et al. (2017): Small RNA-seq during acute maximal exercise reveal RNAs involved in vascular inflammation and cardiometabolic health: brief report. Am J Physiol Heart Circ Physiol 313, H1162–H1167

Shi J, Bei Y, Kong X, Liu X, Lei Z, Xu T, Wang H, Xuan Q, Chen P, Xu J, et al. (2017): miR-17-3p Contributes to Exercise-Induced Cardiac Growth and Protects against Myocardial Ischemia-Reperfusion Injury. Theranostics 7, 664–676

Silva GJJ, Bye A, el Azzouzi H, Wisløff U (2017): MicroRNAs as Important Regulators of Exercise Adaptation. Prog Cardiovasc Dis 60, 130–151

Singh AM, Neva JL, Staines WR (2016): Aerobic exercise enhances neural correlates of motor skill learning. Behav Brain Res 301, 19–26

Stock S, Ihle P, Simic D, Rupprecht C, Schubert I, Lappe V, Kalbe E, Tebest R, Lorrek K (2018): Prävalenz von Demenz bei Versicherten mit und ohne deutsche Staatsangehörigkeit: Eine Studie auf Basis von Daten der gesetzlichen Krankenversicherung. Bundesgesundheitsbl 61, 404–411

Tapia-Rojas C, Aranguiz F, Varela-Nallar L, Inestrosa NC (2016): Voluntary Running Attenuates Memory Loss, Decreases Neuropathological Changes and Induces Neurogenesis in a Mouse

Model of Alzheimer's Disease: Running Prevents Neuropathological Changes in AD Mice. Brain Pathol 26, 62–74

Taurino C, Miller WH, McBride MW, McClure JD, Khanin R, Moreno MU, Dymott JA, Delles C, Dominiczak AF (2010): Gene expression profiling in whole blood of patients with coronary artery disease. Clin Sci 119, 335–343

Toyama K, Spin JM, Deng AC, Huang TT, Wei K, Wagenhäuser MU, Yoshino T, Nguyen H, Mulorz J, Kundu S, et al. (2018): MicroRNA-Mediated Therapy Modulating Blood–Brain Barrier Disruption Improves Vascular Cognitive Impairment. Arterioscler Thromb Vasc Biol 38, 1392–1406

van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999): Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96, 13427–13431

Vasudevan S (2012): Posttranscriptional Upregulation by MicroRNAs: Posttranscriptional Upregulation by MicroRNAs. Wiley Interdiscip Rev RNA 3, 311–330

Vasudevan S, Tong Y, Steitz JA (2007): Switching from repression to activation: microRNAs can up-regulate translation. Science 318, 1931–1934

Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT (2011): MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 13, 423–433

von Arnim CAF, Bartsch T, Jacobs AH, Holbrook J, Bergmann P, Zieschang T, Polidori MC, Dodel R (2019): Diagnosis and treatment of cognitive impairment. Z Gerontol Geriat 52, 309–315

WHO (2017): Mhgap intervention guide for mental, neurological and substance-use disorders in non-specialized health settings. 2nd Edition, https://www.who.int/publications/i/item/mhgap-intervention-guide---version-2.0; accessed on 15.05.2021.

WHO (2019): Risk reduction of cognitive decline and dementia: WHO guidelines. https://www.who.int/mental_health/neurology/dementia/guidelines_risk_reduction/en/; accessed on 15.05.2021

Winter J, Jung S, Keller S, Gregory RI, Diederichs S (2009): Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol 11, 228–234

Ye X, Luo H, Chen Y, Wu Q, Xiong Y, Zhu J, Diao Y, Wu Z, Miao J, Wan J (2015): MicroRNAs 99b-5p/100-5p Regulated by Endoplasmic Reticulum Stress are Involved in Abeta-Induced Pathologies. Front Aging Neurosci 7, 210

Zhang H, Kolb FA, Jaskiewicz L, Westhof E, Filipowicz W (2004): Single processing center models for human Dicer and bacterial RNase III. Cell 118, 57–68

Zhang T, Brinkley TE, Liu K, Feng X, Marsh AP, Kritchevsky S, Zhou X, Nicklas BJ (2017): Circulating MiRNAs as biomarkers of gait speed responses to aerobic exercise training in obese older adults. Aging (Albany NY) 9, 900–913

Zhao Y, Ma Z (2016): Swimming training affects apoptosis-related microRNAs and reduces cardiac apoptosis in mice. Gen Physiol Biophys 35, 443–450

Zhao Y, Zhang A, Wang Y, Hu S, Zhang R, Qian S (2019): Genome-wide identification of brain miRNAs in response to high-intensity intermittent swimming training in Rattus norvegicus by deep sequencing. BMC Mol Biol 20, 3

Zhu Y, Li C, Sun A, Wang Y, Zhou S (2015): Quantification of microRNA-210 in the cerebrospinal fluid and serum: Implications for Alzheimer's disease. Exp Ther Med 9, 1013–1017

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