Lactate mediates the effects of exercise on learning and memory through SIRT1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF)

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https://doi.org/10.1523/JNEUROSCI.1661-18.2019

Received: 2 July 2018
Revised: 14 December 2018
Accepted: 13 January 2019
Published: 28 January 2019


Conflict of Interest: The authors declare no competing financial interests.

This work was supported by grants from the Lebanese American University School of Arts and Sciences and Graduate Research Fund. The TRKb and p-TRKb Antibodies are a kind gift from Dr. Moses V. Chao. SFS conceived the study, performed experiments and wrote the manuscript. LEH performed the experiments with the help of MK, VZ, RAA, NE, NK, REG, PN, MB, PI, NB, VJ and JY. JSS provided valuable advice on Sirtuin function and reagents and helped write the manuscript.

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Cite as: J. Neurosci 2019; 10.1523/JNEUROSCI.1661-18.2019

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Abbreviated title: Lactate mediate exercise’s effects on learning

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Number of Figures: 8
Number of words Abstract: 188
Number of words Introduction: 650
Number of words Discussion: 1161

Acknowledgements: This work was supported by grants from the Lebanese American University School of Arts and Sciences and Graduate Research Fund. The TRKb and p-TRKb Antibodies are a kind gift from Dr. Moses V. Chao. SFS conceived the study, performed experiments and wrote the manuscript. LEH performed the experiments with the help of MK, VZ, RAA, NE, NK, REG, PN, MB, PI, NB, VJ and JY. JSS provided valuable advice on Sirtuin function and reagents
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**Abstract**

Exercise promotes learning and memory formation. These effects depend on increases in hippocampal BDNF, a growth factor associated with cognitive improvement and the alleviation of depression symptoms. Identifying molecules that are produced during exercise and that mediate hippocampal Bdnf expression will allow us to harness the therapeutic potential of exercise. Here, we report that an endogenous molecule produced during exercise in male mice induces the Mus musculus Bdnf gene and promotes learning and memory formation. The metabolite lactate, which is released during exercise by the muscles, crosses the blood brain barrier and induces Bdnf expression and TRKB signaling in the hippocampus. Indeed, we find that lactate-dependent increases in BDNF are associated with improved spatial learning and memory retention. The action of lactate is dependent on the activation of the Sirtuin1 deacetylase. SIRT1 increases the levels of the transcriptional coactivator PGC1a and the secreted molecule FNDC5, known to mediate Bdnf expression. These results reveal an endogenous mechanism to explain how physical exercise leads to the induction of BDNF, and identify lactate as a potential endogenous molecule that may have therapeutic value for central nervous system diseases in which BDNF signaling is disrupted.

**Significance Statement:**
It is established that exercise promotes learning and memory formation and alleviates the symptoms of depression. These effects are mediated through inducing *Bdnf* expression and signaling in the hippocampus. Understanding how exercise induces *Bdnf* and identifying the molecules that mediate this induction will allow us to design therapeutic strategies that can mimic the effects of exercise on the brain especially for patients with CNS disorders characterized by a decrease in *Bdnf* expression and who can not exercise because of their conditions. We identify lactate as an endogeneous metabolite that is produced during exercise, crosses the blood brain barrier and promotes hippocampal dependent learning and memory in a BDNF-dependent manner. Our work identifies lactate as a component of the "exercise pill".

**Introduction:**

Exercise attenuates the symptoms of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Adlard et al., 2005b; Nichol et al., 2007; Tajiri et al., 2010; Real et al., 2013) as well as relieves the symptoms of depression (Russo-Neustadt et al., 2000; Duman et al., 2008). Exercise mediates these beneficial responses by inducing neurogenesis (van Praag et al., 1999), and improving learning and memory formation (Vaynman et al., 2004; Vaynman et al., 2006; Berchtold et al., 2010). The positive changes observed in the brain after exercise are mediated by the induction of brain-derived neurotrophic factor (BDNF) expression in the hippocampus (Neeper et al.; Oliff et al., 1998; Tong et al., 2001; Berchtold et al., 2005) and the activation of its tropomyosin kinase receptor B (TRKB) receptor (Vaynman et al., 2004; Real et al., 2013; Parrini et
Indeed, blocking BDNF signaling in the hippocampus attenuates exercise-induced learning and memory formation (Vaynman et al., 2004; Garcia-Mesa et al., 2014; Kim and Leem, 2016).

BDNF is highly expressed in the brain where it regulates neuronal survival, growth and differentiation during development. BDNF also mediates spine formation and neuronal plasticity as well as promotes learning and memory (Mitre et al., 2017). Indeed, alterations in BDNF/TRKB signaling are observed in a variety of central nervous system (CNS) disorders (Gupta et al., 2013; Mitre et al., 2017). Considering the important role that BDNF plays in mediating brain health, identifying novel molecules and pathways that induce BDNF is of immediate therapeutic relevance.

It has long been established that exercise increases BDNF levels and signaling in the hippocampus to enhance learning and memory formation and ameliorate the symptoms of diseases (Cotman et al., 2007; Wrann et al., 2013; Sleiman et al., 2016), however, the molecular pathways responsible for the exercise-mediated BDNF induction have been elusive. There has been some recent progress in elucidating these pathways. We have shown that a ketone body, β-hydroxybutyrate (DBHB) produced in the liver during exercise, is released into the blood where it accumulates in the hippocampus and induces Bdnf expression by acting as a direct class I HDAC inhibitor. By inhibiting HDAC2 and HDAC3 and preventing their recruitment to the Bdnf promoter I, DBHB induces Bdnf expression and mediates synaptic plasticity (Sleiman et al., 2016). Another mechanism by which exercise induces hippocampal Bdnf expression results from
the induction of the transcriptional coactivator PGC1a and the estrogen-related
receptor ERRa. This transcriptional activating complex induces the expression of
the myokine gene *Fndc5*. FNDC5 activates hippocampal *Bdnf* expression
(Wrann et al., 2013). It is not yet known how FNDC5, a protein that is cleaved
and secreted, is capable of activating hippocampal *Bdnf* gene expression to
promote learning and memory formation.

In this work, we endeavored to identify novel exercise-produced molecules that
could induce hippocampal *Bdnf* gene expression. Identifying such endogenous
“exercise factors” transmitted from peripheral organs through the blood to the
CNS where they can mediate brain health may be crucial for harnessing the full
therapeutic potential of exercise.

One molecule that is released after exercise by the muscle is lactate. Lactate is
released into the blood and taken up by the liver, where it is converted back to
pyruvate, and later to glucose or glycogen depending on cellular needs. Lactate
also crosses the blood-brain barrier (BBB) via endothelial monocarboxylate
transporters (MCTs) (Pierre and Pellerin, 2005; E et al., 2013) and serves as an
energy source for the brain (Quistorff et al., 2008) and as a neuroprotective factor
(Berthet et al., 2009; Bezzi and Volterra, 2011; Newman et al., 2011; Suzuki et
al., 2011). Because of these effects, we hypothesized that lactate is an exercise
factor that induces hippocampal *Bdnf* expression and mediates exercise’s effects
on learning and memory. In this work, we show that voluntary exercise induces
lactate accumulation in the hippocampus, where it promotes learning and
memory formation by inducing Bdnf expression through silent information regulator 1 (SIRT1)-dependent induction of the PGC1a/FNDC5 pathway.

Materials and Methods

Animal housing and lactate injections
Adult male C57BL/6 mice were housed in cages and divided according to the experimental groups: saline or lactate receiving. The mice were provided with food and water ad libitum and maintained on a 12-h light-dark cycle. Male mice were intraperitoneally injected with different lactate concentrations (117mg/Kg or 180mg/Kg) alone or in combination with the TRK inhibitor CEP701 (3mg/Kg) or with saline. Mice were sacrificed 1h or 4hrs after injection time and the brains were dissected on dry ice and the hippocampi were collected and stored at -80°C for later analysis. Animal care and use was in accordance with the guidelines set by the National Institutes of Health and the Lebanese Ministry of Health and as approved by the ACUC.

Exercise paradigm: Male mice were individually housed with food and water ad libitum and maintained on a 12-h light-dark cycle. They were divided into 2 groups: Sedentary animals or exercising animals. The exercising animals were housed with free access to a running wheel. Animals were sacrificed after 30 days and hippocampi were collected and immediately frozen on dry ice. Animal care and use was in accordance with the guidelines set by the National Institutes of Health and the Lebanese Ministry of Health. In the experiments that tested the effect of lactate transporter inhibition on exercise-induced hippocampal Bdnf expression, animals received intraperitoneal injections of ARC-155858 (50nM/mouse) once daily from on
exercise days 26-28, and twice daily within 5 hours time interval on days 29 and 30.

Animals were sacrificed after 30 days and hippocampi were collected and immediately frozen on dry ice. In the experiments that tested the effect of lactate transporter inhibition on exercise-induced spatial learning, animals received intraperitoneal injections of ARC-155858 (50nM/mouse) once daily from on exercise days 26-30, and twice daily for the duration of the water maze test.

**Cell culture:** Immature primary cortical, hippocampal and mixed (cortical/hippocampal) neurons were obtained from C57BL/6 mice [embryonic day 17 (E17)] as previously described (Ratan et al., 1994a, b). Mature cortical neurons were maintained in Neurobasal media (Invitrogen) supplemented with B27, and Glutamax (Invitrogen).

**Cell treatment:** Primary neurons were isolated as described above and 1 million cells were plated in six well plates. On Day 6, cells were treated with different concentrations of lactate (Sigma) for 1 or 4 hours. Lactate was prepared as 0.62M stock in PBS and used at a final concentration of 20mM. Sirtinol (Sigma) was prepared as 10mM stock in DMSO and used at a final concentration of 50 μM.

**RNA extraction and Real Time PCR:** Total RNA was prepared from primary cortical neurons or hippocampi using the Rneasy Plus Mini RNA extraction kit (Qiagen) or NucleoSpin RNA II Kit (Clontech) according to the manufacturer’s protocol. Reverse transcription was performed using iScript cDNA Reverse transcription kit (Bio-rad) according to the manufacturer’s protocol. Real-time PCRs were performed using standard PCR protocol, with Sybr green dye (BioRad).
Details of the Primers used are provided below:

Primer sequence (5'-3'):

**Bdnf pI:**
- Fwd: 5’- CAGGACAGCAAGCCACAAT
- Rev: 5’-GCCTTCATGCAACGGAAGTA

**Sirt 1:**
- Fwd: AAAGGAATTTGTTATTTATCAGAG
- Rev: TTGTGTTTTTCTTCCACACA

**Pgc1a:**
- Fwd: AAAGGAATTTGTTATTTATCAGAG
- Rev: TTGTGTTTTTCTTCCACACA

**Fndc5:**
- Fwd: 5’-CTCTCTGCTCCTCCCTGTTC
- Rev: 5’-CCGACCTTCACCATTTTGTC

**Arc:**
- Fwd: 5’-TACCGTTAGCCCCTATGCCATC
- Rev: 5’-TGATATTGCTGAGCCTCAACTG

**Zif268:**
- Fwd: 5’-TATGAGCACCTGACCAGGTCC
- Rev: 5’-CGAGTCGTTTGGGTGGCTGGAATAC

Protein Extractions: Total cell proteins were prepared by lysing cells in RIPA-B (1% Triton X-100, 1% SDS, 50mM Tris-Cl, pH 7.4, 500mM NaCl and 1mM EDTA) in the presence of protease inhibitors (Sigma), the proteasome inhibitor MG-132 (Sigma) and phosphatase inhibitors (Sigma) followed by benzonase nuclease (Sigma) digestion for 15 minutes. Nuclear proteins were prepared by homogenizing and lysing hippocampi first in buffer A (10 mm HEPES, pH 7.9, 10 mm KCl, 1.5 mm MgCl2, 0.34 m sucrose, 10% glycerol, 0.1% Triton X-100, 1 mm DTT, protease inhibitors, PMSF, MG132, sodium orthovanadate). Next, nuclear proteins were extracted in buffer B (3 mm EDTA, 0.2 mm EGTA, 0.3 m NaCl, 1 mm DTT, protease inhibitors, MG132, phosphatase inhibitors).
Lactate measurements: hippocampal lactate levels were measured using the L-lactate assay kit (Abcam) according to the manufacturer's protocol.

Sirt1 Activity measurements: hippocampal Sirt1 activity was measured from nuclear lysates using the Universal SIRT Activity Assay Kit (Abcam) according to the manufacturer's protocol.

Western blot analysis: Samples were boiled in Laemmli buffer and electrophoresed on Bis-Tris 30% acrylamide gels (Bio-Rad). Proteins were transferred to a PVDF membrane (Bio-Rad) using semi-dry TransBlot Turbo Transfer System (Bio-Rad). Nonspecific binding was inhibited by incubation in blocking buffer (BSA and TBS-Tween20). Antibodies against Trkb (gift from Moses V Chao), phospho-Trkb (gift from Moses V Chao), BDNF (Santa Cruz), ARC (Abcam), ZIF268 (Abcam), PGC1a (Abcam), FNDC5 (Abcam) and β-ACTIN (Sigma) were diluted 1:1000, 1:1500, 1:1000, 1:1000 and 1:5000, respectively in blocking buffer and the membranes were incubated overnight at 4 °C. Secondary antibodies (BioRad) were used at a 1:5000 dilution followed by incubation for 90 minutes at room temperature. Finally, proteins were detected by chemiluminescence on ChemiDoc (Bio-Rad) using Clarity Western ECL Substrate (Bio-Rad) or by SuperSignal™ West Femto Maximum Sensitivity Substrate (Pierce) for PGC1A and BDNF.

Sirt1 short hairpin RNA knockdown:
Five Sirt1(NM_019812) short hairpin RNA (shRNA) clones (Sigma) were used to knockdown the expression of Sirt1 in mixed primary neuronal cultures.
The Sirt1 shRNA clones and a Non-Target shRNA Control Vector (Sigma) were introduced into immature primary mixed neurons (E17) using the Amaxa mouse Neuron Nucleofector kit as directed by the manufacturer (Lonza). On Day 6, SIRT1 knockdown was confirmed by Real-time RT-PCR.

**Morris Water Maze (MWM)**

C57BL/6 male mice (6 weeks) received intraperitoneal injections of saline or lactate and tested in a MWM after 4 hours. All water maze data was recorded using ANY-maze Video Tracking System. The MWM was used as previously described (Morris, 1984). Briefly, mice used visual cues placed on the borders of a swimming pool to reach a hidden platform and escape from the water. Learning was assessed across five days. Prior to learning assessment, mice were introduced into the pool that contains clear water and a visible platform. This training allowed the mice to become familiar with the task. During the learning phase, white paint was added to the water.
and the platform was submerged. Each mouse was subjected to three trials from different starting points. Latency or the time required to reach the platform was recorded every day by the ANY-maze Video Tracking System. On the last day of the experiment, the platform was removed and each mouse was reintroduced into the water and the time spent in the quadrant that previously contained the platform (target quadrant) was recorded. The n number is 35, 31 and 34 for control, lactate 117mg/Kg and 180mg/Kg, respectively.

**Statistical Analysis:** unpaired t-test, 1way or 2way ANOVA followed by the Dunnett or Bonferroni post tests respectively were used to measure statistical significance. p<0.05 was considered to be statistically significant. All graphs are presented as mean +/- SEM.

**Results**

Voluntary exercise promotes hippocampal Bdnf expression as well as learning and memory formation in a lactate dependent manner

To assess whether exercise induces increases in hippocampal lactate concentrations, we subjected mice to a 30-day-long voluntary exercise protocol (Fig. 1A) (Wrann et al., 2013; Sleiman et al., 2016). Exercising mice in this paradigm have significantly higher hippocampal Bdnf expression levels (p=0.001 for exercise versus control, one-way ANOVA) as measured by Real Time RTPCR (Fig. 1C) as well as BDNF signaling (Wrann et al., 2013; Sleiman et al., 2016). Interestingly, mice subjected to voluntary exercise showed a modest, yet significant increase in hippocampal lactate levels as compared to control mice (p=0.0366 and df=17, unpaired t-test) (Fig. 1B). This data is consistent with
previously reported increases in brain and hippocampal lactate levels after different exercise paradigms (Ide et al., 1999; Ide et al., 2000; Ferreira et al., 2007; Dienel, 2012). To assess whether voluntary exercise induces hippocampal Bdnf expression by increasing lactate levels, exercising mice received intraperitoneal injections of the lactate monocarboxylate transporter (MCT1/2) inhibitor, AR-C155858 (Ovens et al., 2010). The Bdnf gene has multiple promoters that generate many transcripts through alternative splicing with a common coding exon (Pruunsild et al., 2011). We focused on the Bdnf gene promoter I (pI) since it is a neuronal activity-dependent (Tabuchi et al., 2002) and exercise-dependent promoter (Tong et al., 2001; Sleiman et al., 2016). As expected, exercising mice have significantly higher hippocampal Bdnf promoter I (Fig. 1C), but not promoter IV or coding (Fig. 1D) expression levels compared to control as measured by Real Time RT-PCR. The increase in promoter I expression was abolished in exercising mice that received the lactate transporter inhibitor, AR-C155858 (p=0.001 for exercise versus control and p=0.4778 for exercise+AR-C155858 versus control; one-way ANOVA followed by Dunnett’s post test) (Fig. 1C). These data demonstrate that lactate that is released in the peripheral organs during voluntary exercise, crosses the BBB and accumulates in the hippocampus where it is important for exercise’s effect on Bdnf induction. Intra-peritoneal delivery of lactate induces Bdnf expression and signaling in the hippocampus

To assess whether lactate, a metabolite that is increased in the blood by exercise (Smith et al., 1997; Ide et al., 1999; Ide et al., 2000; Ferreira et al., 2007;
Meek et al., 2009; Dienel, 2012), directly enhances Bdnf gene expression in the hippocampus, we injected mice intraperitoneally with either saline or lactate (117 or 180 mg/Kg). Animals were sacrificed one hour after the injections and tissues were collected for analysis. The amount of lactate that was injected yields 13mM or 20mM lactate concentrations in the blood. These concentrations are consistent with lactate plasma levels reported after exercise. Indeed, exercise induces an increase in plasma lactate concentrations that can reach up to 30mM (Dienel, 2012) as well as a concomitant increase in brain and hippocampal lactate concentrations (Ide et al., 1999; Ide et al., 2000; Ferreira et al., 2007; Dienel, 2012). The two concentrations we used yielded modest increases in hippocampal lactate levels (Fig. 2A) (p=0.003 for lactate 117mg/Kg versus control; one-way ANOVA followed by Dunnett’s post-test) very similar to the increases that were observed in the exercising mice (Fig. 1B). To determine the effects of lactate on Bdnf expression in the hippocampus, we performed Real time RTPCR. Our results showed that like exercise (Fig. 1C), lactate significantly induced Bdnf promoter I expression in the hippocampus (p=0.0316 for lactate 117mg/Kg versus control and p=0.0564 for lactate 180mg/Kg versus control, one-way ANOVA followed by Dunnett’s post-test) (Fig. 2B), but not Bdnf promoter IV or coding expression (Fig. 2C). Western blot analysis of hippocampal tissue isolated from animals receiving lactate showed a significant increase in BDNF protein levels (p=0.0438, and df=9 as measured by unpaired t-test) and TRKB phosphorylation as compared to control mice (p=0.000052 and df=4 as measured by unpaired t-test) (Fig. 2D, 2E and F) as well as an increase in the protein levels of the synaptic plasticity genes ARC and ZIF268.
(ZIF268 p=0.0196, df=9 for lactate 180mg/Kg versus control; ARC p=0.0303, df=7 for lactate 180mg/Kg versus control as measured by one-way ANOVA followed by the Dunnett’s post test) (**Fig. 2G and H**). Taken together, the data is consistent with systemic delivery of lactate activating BDNF signaling pathways in the hippocampus. Interestingly, inhibiting the MCT1/2 lactate transporters abolished the lactate mediated induction of hippocampal Bdnf expression emphasizing the importance of ability of lactate to cross the BBB (**Fig. 2I**). Interestingly, the ability of lactate to induce Bdnf promoter I, but not promoter IV or coding expression was not restricted to the hippocampus but was also observed in the cortex (p=0.0367, df=6 as measured by unpaired t-test) (**Fig. 3A**). These results led us to test whether lactate-mediated induction of Bdnf expression is neuron-specific.

**Lactate induces Bdnf and synaptic plasticity gene expression in primary neurons**

In order to test whether lactate can induce the expression of Bdnf in neurons, we treated primary neuronal cultures with lactate for one hour, extracted neuronal RNA and performed real time RTPCR. We found that lactate significantly induced Bdnf promoter I expression in primary hippocampal (p=0.0073 and df=9 for lactate 20mM versus control) (**Fig. 3A**) and cortical neuronal cultures (p=0.0014 and df=4 for lactate 20mM versus control; unpaired t-test) (**Fig. 3B**). In addition, lactate also induced the expression of synaptic plasticity genes such as Arc and Zif268/Egr1 (for Arc, p=0.0014 and df=5 for lactate 20mM versus control and for Zif268, p=0.0039 and df=6 for lactate 20mM versus control) (**Fig. 3B**). We also confirmed that lactate increases the protein levels of BDNF (p=0.0199, df=4 for...
lactate 20mM versus control) (Fig. 3D. and F), ZIF268 (p<0.0001, df=2 for lactate 20mM versus control) (Fig. 3D. and G) and ARC (p=0.0483, df=4 for lactate 20mM versus control) (Fig. 3E. and H). These results suggest that lactate can specifically induce the expression of synaptic plasticity genes in neuronal cells. For this reason, we decided to test whether the lactate-mediated induction of synaptic plasticity genes and particularly BDNF/TRKB signaling correlates with learning and memory phenotypes.

Lactate enhances learning and memory formation

In order to test whether the lactate-mediated induction of Bdnf expression is responsible for enhancing learning and spatial memory formation, we performed Morris water maze (MWM) experiments. The MWM is a spatial learning task that requires mice to locate a hidden platform in an opaque pool of water using visual cues. Acquisition of spatial learning in both control mice and lactate-injected mice was observed as reduced latency to reach the hidden platform by day 5. Mice receiving lactate (117mg/Kg and 180mg/Kg) significantly outperformed the control mice (Fig. 4A). To assess reference memory, we performed a probe trial 24 h after the last training session (day 6), during which the platform was removed. As expected, lactate-injected mice showed significant enhancement of memory recall, as indicated by increased time spent in the target quadrant (Fig. 4B). In this test, we only observed significant enhancement with the 180mg/Kg lactate dose. Both control and lactate-injected mice had similar swim speed during training days 1–5 (Fig. 4C). These results show that lactate did not affect mouse motility nor swimming ability. To assess whether the lactate-mediated activation of the
BDNF/TRKB pathway is responsible for enhanced memory formation, we injected mice with lactate (180mg/Kg) in combination with a TRK inhibitor, CEP701 (Obeid et al., 2014). Mice receiving this combinatorial treatment did not show a significant enhancement of memory recall (Fig. 4D). Taken together, our results are consistent with the model that exercise induces an increase in lactate concentration in the blood and hippocampus, which in turn mediates the induction of synaptic plasticity genes such as Bdnf, leading to enhanced spatial learning and memory retention. In order to verify whether indeed exercise-induced learning is lactate-dependent, we injected exercising mice with the lactate transporter inhibitor AR-C155858 and tested their ability to navigate the MWM. These mice exhibited significantly worsened learning curves as compared to exercise mice injected with saline (Fig. 4E). We were next interested in elucidating the mechanism of action of lactate and specifically how lactate can mediate Bdnf induction in the hippocampus.

Lactate induces Bdnf expression in a SIRT1 dependent mechanism

Exercise is accompanied by increases in energy requirements and changes in the levels of high energy molecules such as ATP and the reduced co-enzyme NADH. One class of enzymes whose activity is dependent on NAD⁺ levels is the Sirtuins or class III histone deacetylases (HDAC). This class of HDACs includes seven members (SIRT1-7) and is not only involved in the deacetylation of histones, but also many cellular proteins including transcription factors. Among the members of this family, only SIRT1, SIRT6 and SIRT7 are resident nuclear proteins. Interestingly, voluntary exercise significantly induced the hippocampal expression of only Sirt1 at both the mRNA (p=0.026 and df=6; unpaired t-test) (Fig. 5A) and
protein levels (p=0.0456 and df=6; unpaired t-test). (Fig. 5B and C). Even though exercise induced Sirt7 mRNA as measured by Real Time RTPCR, it did not significantly induce SIRT7 protein levels (Data not shown). Since lactate is produced and removed in reactions that directly affect the NAD\(^+\)/NADH ratio, we decided to evaluate if lactate also affected SIRT7 levels. We injected mice intraperitoneally with saline or lactate and examined the protein levels of SIRT1 in the hippocampus. We found that systemic delivery of lactate significantly increased the hippocampal levels of SIRT1 (p=0.0077 and df=9; unpaired t-test) (Fig. 5D and E). Interestingly, like exercise, lactate also induced Sirt7 mRNA as measured by Real Time RTPCR, but did not significantly induce SIRT7 protein levels (Data not shown). We next tested whether exercise and lactate affected SIRT1 deacetylase activity. We found that both exercise (p= 0.05 and df=3) and intraperitoneal injections of lactate p=0.059 and df= 3) increased SIRT1 activity in hippocampal nuclear proteins (Fig. 5F). To evaluate if the lactate-mediated induction of Bdnf expression is SIRT1 dependent, we used shRNAs to knockdown Sirt1 expression as well as a SIRT1 inhibitor, sirtinol, and tested whether decreased SIRT1 levels and activity abolished the lactate-mediated Bdnf induction in primary neurons. Indeed, while lactate increased Bdnf promoter I mRNA expression in cells expressing the scrambled shRNA (Ctrl shRNA), it failed to increase Bdnf promoter I mRNA expression in cells expressing the Sirt1 ShRNA (Fig 4G and H). In addition, the lactate-induced Bdnf promoter I mRNA expression was lost upon co-treatment of neurons with lactate and sirtinol (p=0.0273 for control versus lactate, p=0.0087 for sirtinol versus lactate and p=0.0130 for lactate+sirtinol versus lactate; one-way ANOVA followed by the...
Dunnett’s post-test) (**Fig. 5I**). These results suggest that lactate activates Bdnf expression through a SIRT1-dependent mechanism.

**Lactate induces Bdnf expression through SIRT1-dependent induction of PGC1a levels and in turn Fndc5 expression**

It was previously reported that voluntary exercise induces the expression of the transcriptional coactivator Pgc1a and Erra mRNA in the hippocampus. PGC1a and ERRa, in turn, coordinate to activate the hippocampal expression of the myokine Fndc5, which can induce Bdnf expression in the hippocampus (Wrann et al., 2013; Wrann, 2015). However, the mechanism by which the hippocampal induction of this pathway occurs in response to exercise has not been deciphered.

We suspected that lactate could serve as the missing exercise factor. Indeed, one possible explanation for the effects of exercise is that it induces lactate accumulation in the hippocampus, which in turn activates SIRTs. SIRTs induce the PGC1a/FNDC5 pathway and lead to the induction of hippocampal Bdnf expression and enhanced learning and memory. To address this hypothesis, we first sought to confirm and assess whether voluntary exercise and intraperitoneal injections of lactate induce hippocampal PGC1a protein levels. Indeed, western blot analysis reveals that both voluntary exercise (p=0.05, df=4; unpaired t-test) and intraperitoneal injections of lactate (p=0.0436 and df=4; unpaired t-test) significantly increase hippocampal PGC1a protein levels (**Fig. 6A-D**). To determine whether the lactate-mediated induction of Bdnf represents a SIRT1-dependent engagement of the hippocampal PGC1a/FNDC5 pathway, we treated primary neurons with lactate alone or combined with the sirtinol and assessed PGC1a protein levels. Western
blot analysis reveals that lactate induced PGC1a protein levels, and this induction is
lost upon combinatorial treatment of lactate and sirtinol (p=0.0007 for control versus
lactate, p=0.002 for lactate+ sirtinol versus lactate; one-way ANOVA followed by
Dunnett’s post-test). (Fig. 6E and F). These results suggest that both lactate-
mediated induction of PGC1a protein and BDNF expression are SIRT-dependent.

Finally, it is well-established that exercise-induced PGC1a mediates hippocampal
eexpression of FNDC5, which in turn activates Bdnf expression (Wrann et al., 2013;
Wrann, 2015). We observed that like exercise (mRNA: p=0.0183 and df=5; protein:
p=0.0347 and df=4; unpaired test) (Fig. 7 A and C-D), intraperitoneal injections of
lactate induce hippocampal Fndc5 mRNA expression as measured by Real Time
RTPCR (p=0.0152 and df=8; unpaired t-test) (Fig. 7B), and protein levels (p=0.0112
and df=4; unpaired t-test) (Fig. 7E and F). Indeed, we found that while lactate
increased Fndc5 mRNA expression in cells expressing the scrambled shRNA (Ctrl
shRNA), it failed to increase Bdnf promoter I mRNA expression in cells expressing
the Sirt1 shRNA (Fig 7G). Taken together, these results are consistent with the
model that exercise can promote learning and memory by inducing hippocampal
Bdnf expression through lactate-mediated activation of the SIRT1/PGC1a/FNDC5
pathway (Fig. 8).

Discussion

These results provide a link between running exercise, lactate, SIRT1 and
Bdnf gene expression. Previous work showed that lactate that is supplied to
neurons by astrocytes can promote long term potentiation (LTP) (Skriver et al.,
2014) and is important for memory formation (Newman et al., 2011; Suzuki et al.,
Indeed, lactate activates the expression of synaptic plasticity genes by activating NMDA and ERK signaling cascades (Yang et al., 2014). In addition, lactate reduces glutamate-induced toxicity in the cortex (Ros et al., 2001) and protects against ischemic insults both in vitro (Berthet et al., 2012) and in middle cerebral artery occlusion stroke models (Berthet et al., 2009; Berthet et al., 2012; Castillo et al., 2015). The neuroprotective effect of intravenous administration of lactate against ischemic damage suggests that lactate may serve as a potential inexpensive therapeutic strategy for stroke (Berthet et al., 2009; Berthet et al., 2012). It is highly conceivable that lactate might act to increase the levels of BDNF, which can promote learning and memory (Mu et al., 1999; Cirulli et al., 2004) as well as neuroprotection (Beck et al., 1994; Zuccato and Cattaneo, 2009; Zhao et al., 2017).

It has long been established that physical exercise induces hippocampal Bdnf expression (Neeper et al.; Oliff et al., 1998; Tong et al., 2001; Cotman and Berchtold, 2002; Adlard and Cotman, 2004; Adlard et al., 2004; Adlard et al., 2005a; Berchtold et al., 2005) and that this induction is necessary for cognitive processes such as learning and memory (Vaynman et al., 2004; Garcia-Mesa et al., 2014; Kim and Leem, 2016). We have only recently started deciphering the molecular pathways linking the exercise-induced metabolic changes in the liver and muscle to hippocampal Bdnf expression and increased cognition. It is clear though that the link involves multiple endogenous “exercise factors” that are released in the blood, can cross the BBB and accumulate in the hippocampus where they engage signaling pathways that activate Bdnf expression. One previously-identified exercise factor is...
beta-hydroxybutyrate (DBHB), a ketone body produced by the liver during exercise that accumulates in the hippocampus and induces $Bdnf$ expression through HDAC inhibition (Sleiman et al., 2016). In this work, we identified lactate as a novel endogenous metabolite that links exercise to hippocampal $Bdnf$ expression and to cognition. Lactate is not only supplied to neurons by astrocytes, but is also released into the blood by the muscle during exercise. Lactate crosses BBB through MCTs and reaches multiple brain regions including the hippocampus. During exercise, circulating lactate levels can reach 30 mM, and hippocampal levels also significantly increase (Fig. 1 and Ide et al., 1999; Ide et al., 2000). Interestingly, exercise increases $Mct2$ levels in the hippocampus and this increase is correlated with increases in BDNF and TrkB signaling (Takimoto and Hamada, 2014). This observation is consistent with the measured increases in lactate (Fig. 2) that uses the $Mct2$ to reach the hippocampus where it can mediate $Bdnf$ expression (Fig. 2) (Sleiman et al., 2016). Indeed, lactate levels increase in the hippocampus after exercise (Fig. 1 and Ide et al., 1999; Ide et al., 2000). Like exercise, systemic delivery of lactate through intraperitoneal injections induces hippocampal $Bdnf$ expression and signaling and can mediate learning and memory formation (Fig. 1-4).

Our results are consistent with the finding that lactate released from exercising muscles mediates cerebral angiogenesis through the activation of the lactate receptor HCAR1 (Morland et al., 2017). Interestingly, we found that lactate activates the NAD$^+$-dependent histone deacetylase SIRT1 (Fig. 5), which in turn engage the previously-identified hippocampal PGC1a/FNDC5 pathway to induce $Bdnf$ expression (Fig. 6-7). Exercise has been shown to increase $Bdnf$ levels through the
induction of hippocampal expression of Fndc5, a PGC1α and ERRα-dependent myokine (Wrann et al., 2013; Wrann, 2015). How exercise mediates the induction of PGC1α and ERRα in the hippocampus has not been clearly established. Like exercise, lactate modulates the redox status of neurons by altering the NAD+/NADH ratio (Koltai et al., 2010) which leads to the activation of SIRT1. We observed that the exercise-mediated induction of hippocampal PGC1α (Fig. 5) and in turn FNDC5 (Fig. 6) expression is dependent on SIRT1 activity (Fig. 5).

A common theme that emerges from our data is the convergence of the action of the identified exercise factors on protein acetylation. DBHB acts as a HDAC2/3 inhibitor and mediates Bdnf induction by promoting histone acetylation at its promoter, whereas lactate activates SIRT1 that deacetylates the transcriptional coactivator PGC1α. This promotes its activity (Gerhart-Hines et al., 2007; Canto et al., 2009) and allows it to induce gene expression. This is not surprising considering that histone acetylation is a key regulator of memory consolidation in the hippocampus (Alarcon et al., 2004; Korzus et al., 2004; Levenson et al., 2004).

Indeed, CREB binding protein (CBP) has been shown to be necessary for long-term memory formation (Guan et al., 2002; Korzus et al., 2004; Chen et al., 2010; Giralt et al., 2012). Introduction of a CBP activator that can cross the BBB promotes neurogenesis and increases memory duration (Chatterjee et al., 2013). In addition, CBP gene transfer rescues learning and memory deficits in an AD mouse model through the induction of BDNF (Caccamo et al., 2010). Moreover, pharmacological inhibition of HDACs promotes learning and memory formation (Levenson et al., 2004; Vecsey et al., 2007; Stefanko et al., 2009; Penney and Tsai, 2014). Indeed,
how multiple HDAC isoforms affect learning and memory has been elucidated. For example, HDAC2, negatively regulates hippocampal-dependent memory (Guan et al., 2009; Yamakawa et al., 2017). Recently, it was found that the enzyme acetyl-CoA synthetase 2 (ACSS2) binds to promoters of neuronal activity and memory-related genes where it locally catalyzes the production of acetyl coenzyme A (Acetyl CoA) from acetate, coenzyme A (CoA) and ATP. The Acetyl group of acetyl CoA is transferred by HATs to the local histones leading to unwinding of chromatin and activation of gene expression (Mews et al., 2017; Watson and Tsai, 2017). Because of the ability of exercise factors to modulate protein acetylation, it would be interesting to determine whether other metabolites including acetyl CoA that can affect protein acetylation play important roles in the positive effects of exercise on learning and memory.

In this paper, we provide evidence that an endogenous molecule, lactate, that crosses the BBB, is increased by exercise to enhance BDNF signaling in the hippocampus and in turn promote learning and memory (Fig. 8). These results support the hypothesis that the elusive factors that mediate the positive effects of exercise on the brain serve the dual purpose of an energy fuel and epigenetic modulators that mediate their effects by altering hippocampal gene expression. Continued identification of additional such factors is of relevance to people afflicted with neurodegenerative diseases or depression who are likely to benefit from the ability of exercise to stimulate BDNF. Interestingly, there is recent evidence the lactate can promote resilience to stress in chronic social defeat mouse models (NK, REG, SFS unpublished data) as well as serve as an antidepressant (Carrard et al., 2017).
REFERENCES AND NOTES


Figure Legends

Figure 1: Lactate mediates in part the voluntary exercise-mediated induction of hippocampal Bdnf expression and promotion of learning. (A) The exercise paradigm involves 4 weeks of voluntary exercise followed by animal euthanasia and hippocampal isolation. (B) Voluntary exercise for 4 weeks significantly increases hippocampal lactate levels. The number of hippocampi used for each group (control and exercise) is 10 and 9 respectively. * p<0.05 as measured by unpaired t-test; p=0.0366 and df=17. (C) Voluntary exercise significantly induces Bdnf promoter I expression in the hippocampus as measured by real-time RTPCR whereas inhibiting the lactate MCT transporters by AR-C155858 (50nM/mouse) abolishes this induction. The number of hippocampi used for each group (control and exercise and exercise +AR-C155858) is 5 and 2 and 4 respectively. ** p<0.01 as measured by one-way ANOVA followed by Dunnett’s multiple comparison test; p=0.001 for exercise versus control and p=0.4778 for...
exercise+AR-C155858 versus control. (D) Voluntary exercise does not induce $Bdnf$ expression in the hippocampus as measured by real-time RTPCR.

**Figure 2:** Peripheral delivery of Lactate induces hippocampal $Bdnf$ expression and signaling. (A) Intraperitoneal injections of lactate in order to reach equivalent levels to those reported in the blood after exercise lead to increases in hippocampal lactate. This increase is equivalent to the increase in hippocampal lactate observed after exercise. The number of hippocampi used for each group (control and lactate 117mg/Kg or lactate 180mg/Kg) is 10 and 9 and 7 respectively. ** p<0.01 as measured by one-way ANOVA followed by the Dunnett's post-test; p=0.003 for lactate 117mg/Kg versus control. (B) Intraperitoneal injection of lactate (117mg/Kg or 180mg/Kg) similar to levels reported during exercise significantly induces hippocampal $Bdnf$ levels as measured by real-time RTPCR. The expression was analyzed from the hippocampi of 5 control mice, 5 mice receiving 117mg/Kg lactate and 5 mice receiving 180mg/Kg lactate. * p<0.05 as measured by one-way ANOVA followed by the Dunnett's multiple comparison test; p=0.0316 for lactate 117mg/Kg versus control and p=0.0564 for lactate 180mg/Kg versus control. (C) Intraperitoneal injection of lactate (117mg/Kg) does not induce hippocampal $Bdnf$ and coding expression levels as measured by real-time RTPCR. The expression was analyzed from the hippocampi of 4 control mice, and 4 mice receiving 117mg/Kg lactate. (D) Representative western blot image depicting the increase in BDNF
protein levels and in phosphorylation in the BDNF receptor TRKBB (pTRKB) in the hippocampus of control animals as compared to mice receiving 117mg/Kg of lactate. (E) Quantification of the BDNF western blot. Statistical significance was measured by the unpaired t-test * p<0.05; p=0.043 and df=4. (F) Quantification of the pTRKB western blot. Statistical significance was measured by unpaired t-test **** p<0.0001; p=0.000052 and df=4. (G) Representative western blot image depicting the increase after 10 minutes in ZIF268 and ARC protein levels in the hippocampus of control animals as compared to mice receiving 117mg/Kg and 180mg/Kg of lactate. (H) Quantification of the ZIF268 and ARC western blots. Statistical significance was measured by one-way ANOVA followed by the Dunnett's multiple comparison test; * p<0.05, ZIF268 p=0.0196, df=9 for lactate 180mg/Kg versus control; ARC p=0.0303, df=7 for lactate 180mg/Kg versus control. (I) Intraperitoneal injection of lactate (180mg/Kg) along with the lactate MCT transporters AR-C 155858 (50nM/mouse) did not induce hippocampal Bdnf pl expression levels as measured by real-time RTPCR. The expression was analyzed from the hippocampi of 5 control mice, 5 mice receiving 180mg/Kg lactate and AR-C 155858 (50nM/mouse). Significance was measured by unpaired t-test; p=0.9910 and df=8.

Figure 3: Lactate induces exercise-dependent Bdnf pl and activity-dependent gene expression in mouse primary neurons. (A) Intraperitoneal injection of lactate (117mg/Kg) significantly induces cortical Bdnf pl but not pIV or coding expression levels as measured by real-time RTPCR. The expression was
analyzed from the hippocampi of 4 control mice, 4 mice receiving 117mg/Kg lactate * p<0.05 as measured by unpaired t-test; p=0.036, df=6 for lactate 117mg/Kg versus control. (B) Lactate significantly induces Bdnf pl expression in primary hippocampal neurons as measured by real-time RTPCR. Statistical significance was measured by unpaired t-test **p<0.01; p=0.0073 and df=9 for lactate 20mM versus control. The n number used is 5. Each replicate consisted of primary neurons obtained from different cultures and treated with fresh dilutions of the compounds for 1 hour. (C) Lactate significantly induces Bdnf pl and activity dependent gene (Arc and Zif268) expression in primary cortical neurons as measured by real-time RTPCR. Statistical significance was measured by unpaired t-test **p<0.01; for Bdnf pl, p=0.0014 and df=4 for lactate 20mM versus control; for Arc, p=0.0014 and df=5 for lactate 20mM versus control and for Zif268, p=0.0039 and df=6 for lactate 20mM versus control. The n number used is 4. Each replicate consisted of primary neurons obtained from different cultures and treated with fresh dilutions of the compounds for 1 hour. (D and E) Representative western blot images depicting the lactate-mediated increase in BDNF, ZIF268, and ARC protein levels in mixed (cortical and hippocampal) primary neurons. (F) Quantification of the BDNF western blots. Statistical significance was measured by the unpaired t-test; * p<0.05, p=0.0199, df=4. (G) Quantification of the ZIF268 western blots. Statistical significance was measured by the unpaired t-test; * p<0.05, p<0.0001, df=2. (H) Quantification of the ARC western blots. Statistical significance was measured by the unpaired t-test; * p<0.05, p=0.0483, df=4.
Figure 4: Peripheral delivery of lactate promotes learning and memory. (A) Animals were trained for 5 days in a spatial version of the MWM. Animals receiving intraperitoneal injections of lactate (118 mg/Kg and 180mg/Kg) showed significantly reduced escape latency or the time (seconds) required to escape. Results are expressed as Mean +/- SEM. Statistical significance was measured by 2way anova followed by the Bonferroni post-test * p<0.05. (B) Animals receiving intraperitoneal injections of lactate (180mg/Kg) spent significantly more time in the target quadrant during a 60 second probe test performed 1 day after the last training session. Results are expressed as Mean +/- SEM. Statistical significance between the target and the other three quadrants was measured by 1way Anova followed by Dunnett's post-test *** p<0.0001. Statistical significance between the target quadrants in Saline and lactate receiving mice was measured by unpaired t-test; p=0.0056 and df=65. (C) The swimming speeds (meter/seconds) of the animals receiving intraperitoneal injections lactate were similar to those receiving saline indicating that the effects observed in the training and probe test phase were not due to differences in motor behavior between the two groups of animals. (D) Animals receiving intraperitoneal injections of lactate (180mg/Kg) in combination with the TRK inhibitor, CEP 701 (3mg/Kg) did not spend significantly more time in the target quadrant as compared to animals receiving intraperitoneal injections of saline during a 60 second probe test performed 1 day after the last training session. Results are expressed as Mean +/- SEM. The number of animals utilized in each group is 5. Statistical
significance was measured by the unpaired t-test; $p=0.3117$, $df=8$. As expected, animals receiving saline spend significantly more time in the target quadrant. Statistical significance was measured by one-way ANOVA followed by the Dunnett's post-test; $p=0.0002$ for target vs Quadrant 2 (Q2) and 3 (Q3) and $p=0.0014$ for target versus Quadrant 4 (Q4). In contrast, animals receiving lactate and TRK inhibitor CEP701 did not spend significantly more time in the target quadrant as compared to Q3 and Q4. Statistical significance was measured by one-way ANOVA followed by the Dunnett's post-test; $p=0.0295$ for target versus Q2, $p=0.0652$ for target versus Q3 and $p=0.4912$ for target versus Q4. (E) Inhibition of the lactate MCT transporters abolishes exercise-mediated spatial learning in the Morris water maze paradigm. Exercise animals receiving intraperitoneal injections of the lactate MCT transporter inhibitor, AR-C155858 (50nM/mouse) showed significantly increased escape latency or the time (seconds) required to escape as compared to exercise animals receiving intraperitoneal injections of saline. Results are expressed as Mean +/- SEM. Statistical significance was measured by 2way anova followed by the Bonferroni post-test * $p<0.05$

**Figure 5**: Lactate induces Bdnf expression in a SIRT1-dependent manner. (A) Voluntary exercise for 4 weeks significantly induced Sirt1 expression in the hippocampus as measured by real-time RTPCR. The number of animal used for each group (control and exercise) is 3 and 5 respectively. Statistical significance was measured by unpaired t-test * $p<0.05$; $p=0.026$ and $df=6$. (B) Representative
western blot image depicting the increase in SIRT1 protein levels in the hippocampus of control animals as compared to exercising mice (C). Quantification of the SIRT1 western blot. The n number for control and exercise is 4. Statistical significance was measured by unpaired t-test * p<0.05; p=0.0456 and df=6. (D) Representative western blot image depicting the increase in SIRT1 protein levels in the hippocampus of mice receiving 117mg/Kg of lactate as compared to mice receiving saline. (E) Quantification of the SIRT1 western blot. The n number for saline injections is 5 and for lactate injections is 6. Statistical significance was measured by unpaired t-test ** p<0.005; p=0.0077 and df=9. (F) Exercise and lactate (117mg/Kg) induce SIRT1 activity in hippocampal nuclear extracts. Statistical significance was measured by the unpaired t-test *p<0.05; for exercise p= 0.05 and df=3 and for lactate p=0.059 and df= 3. (G) Three shRNA sequences significantly decrease Sirt1 mRNA expression as measured by Real Time RTPCR. Statistical significance was measured by unpaired t-test *p<0.05; for seq 1 p= 0.0351 and df=2, for seq 4 p=0.0045 and df=2 and for seq5 p= 0.05 and df=2. (H) Sirt1 knockdown abolishes the lactate-mediated increase in Bdnf pl expression. Statistical significance was measured by unpaired t-test *p<0.05; p= 0.0497 and df=2 for Ctrl shRNA versus Ctrl shRNA +lactate 20mM (I) Sirtinol, a SIRT1 inhibitor, reversed the lactate-mediated induction of Bdnf pl expression in primary neurons as measured by real-time RTPCR. The n number for controls, lactate (20mM), sirtinol (50μM) and lactate+sirtinol treatments is 6, 4, 6 and 6, respectively. Each replicate consisted of primary neurons obtained from different cultures and treated with fresh...
dilutions of the compounds for 4 hours. Statistical significance was measure by one-way ANOVA followed by the Dunnett’s post-test; * p<0.05 and ** p<0.01; p=0.0273 for control versus lactate, p=0.0087 for sirtinol versus lactate and p=0.0130 for lactate+ sirtinol versus lactate.

**Figure 6:** SIRT1 modulates Bdnf expression through induction of PGC1a protein levels. (A) Representative western blot image depicting the increase in PGC1a protein levels in the hippocampus of control animals as compared to exercising mice (B) Quantification of the PGC1a western blot. Statistical significance was measured by unpaired t-test * p<0.05. p=0.05, df=4 (C) Representative western blot image depicting the increase in PGC1a protein levels in the hippocampus of mice receiving 117mg/Kg of lactate as compared to mice receiving saline. (D) Quantification of the PGC1a western blot. Statistical significance was measured by unpaired t-test * p<0.05; p=0.0436 and df=4. (E) Representative western blot image depicting the increase in PGC1a protein levels in primary neurons treated with lactate (20mM) and the reversal of this increase upon Sirt inhibitor (sirtinol 50uM) co-treatment. These results suggest that the lactate mediated induction of PGC1a protein levels is SIRT1 dependent. (F) Quantification of the PGC1a western blot. Statistical significance was measure by one-way ANOVA followed by the Dunnett’s post-test; * p<0.05; p=0.0007 for control versus lactate, p=0.002 for lactate+ sirtinol versus lactate.
Figure 7: Lactate induces $Fndc5$ levels, a PGC1α-dependent $Bdnf$ inducer. (A) Voluntary exercise for 4 weeks significantly induces $Fndc5$ expression in the hippocampus as measured by real-time RTPCR. The number of animals used for each group (control and exercise) is 3 and 4 respectively. * $p<0.05$ as measured by unpaired t-test; $p=0.0183$ and df=5. (B) Intraperitoneal injections of lactate (117mg/Kg) significantly induced hippocampal $Fndc5$ gene expression levels as measured by Real Time RTPCR. The number of animal used for each group is 5. Statistical significance was measured by unpaired t-test * $p<0.05$; $p=0.0152$ and df=8. (C) Representative western blot image depicting the increase in FNDC5 protein levels in the hippocampus of control animals as compared to exercising mice (D) Quantification of the FNDC5 western blot. Statistical significance was measured by unpaired t-test * $p<0.05$; $p=0.0347$ and df=4. (E) Representative western blot image depicting the increase in FNDC5 protein levels in the hippocampus of mice receiving 117mg/Kg of lactate as compared to mice receiving saline. (F) Quantification of the FNDC5 western blot. Statistical significance was measured by unpaired t-test *$p<0.05$; $p=0.0112$ and df=4. (G) Sirt1 knockdown abolishes the lactate-mediated increase in $Fndc5$ mRNA expression. Statistical significance was measured by unpaired t-test *$p<0.05$; $p=0.0318$ and df=2 for Ctrl shRNA versus Ctrl shRNA +lactate 20mM.

Figure 8: A proposed model by which exercise induces $Bdnf$ expression in the hippocampus. Exercise induces lactate synthesis in the muscle. Lactate is transported through the circulation to the brain. In the hippocampus, lactate...
induces Bdnf expression through SIRT1-dependent induction of PGC1a. PGC1a, in turn, coordinates the increase in Fndc5 expression, which is known to induce Bdnf expression. This induction mediates exercise’s positive effects on memory, cognition and synaptic transmission.
Figure 6

Panel A: Western blot analysis showing PGC1a and ACTIN levels in Control and Exercise conditions.

Panel B: Bar graph comparing Relative PGC1a protein levels between Control and Exercise conditions.

Panel C: Western blot analysis showing PGC1a and GAPDH levels in Control and Lactate (117mg/Kg) conditions.

Panel D: Bar graph comparing Relative PGC1a protein levels between Saline and Lactate (117mg/Kg) conditions.

Panel E: Western blot analysis showing PGC1a and ACTIN levels in Control, Lactate, and Lactate+Sirtinol conditions.

Panel F: Bar graph comparing Relative PGC1a protein levels between Control, Lactate, and Lactate+Sirtinol conditions.
Exercise

Blood

Lactate

Hippocampal neuron

Lactate

Sirt1

Pgc1a

Bdnf

BDNF

Fndc5

Hippocampal neuron

BDNF

Learning and memory

Exercise

Blood

Lactate

Hippocampal neuron

Lactate

Sirt1

Pgc1a

Fndc5

Bdnf