International Journal of
Molecular Sciences

MDPI

Review

Lactate as a Metabolite and a Regulator in the Central Nervous System

Patrizia Proia [1], Carlo Maria Di Liegro [2], Gabriella Schiera [2], Anna Fricano [2] and Italia Di Liegro [3,*]

1 Department of Psychological, Pedagogical and Educational Sciences, Sport and Exercise Sciences Research Unit, University of Palermo, Palermo I-90128, Italy; patrizia.proia@unipa.it
2 Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo (UNIPA), Palermo I-90128, Italy; carlomaria.diliegro@unipa.it (C.M.D.L.); gabriella.schiera@unipa.it (G.S.); anna.fricano@unipa.it (A.F.)
3 Department of Experimental Biomedicine and Clinical Neurosciences (BIONEC), University of Palermo, Palermo I-90127, Italy
* Correspondence: italia.diliegro@unipa.it; Tel.: +39-091-238-97-415 or +39-091-238-97-446

Academic Editor: Irmgard Tegeder
Received: 28 July 2016; Accepted: 25 August 2016; Published: 1 September 2016

Abstract: More than two hundred years after its discovery, lactate still remains an intriguing molecule. Considered for a long time as a waste product of metabolism and the culprit behind muscular fatigue, it was then recognized as an important fuel for many cells. In particular, in the nervous system, it has been proposed that lactate, released by astrocytes in response to neuronal activation, is taken up by neurons, oxidized to pyruvate and used for synthesizing acetyl-CoA to be used for the tricarboxylic acid cycle. More recently, in addition to this metabolic role, the discovery of a specific receptor prompted a reconsideration of its role, and lactate is now seen as a sort of hormone, even involved in processes as complex as memory formation and neuroprotection. As a matter of fact, exercise offers many benefits for our organisms, and seems to delay brain aging and neurodegeneration. Now, exercise induces the production and release of lactate into the blood which can reach the liver, the heart, and also the brain. Can lactate be a beneficial molecule produced during exercise, and offer neuroprotection? In this review, we summarize what we have known on lactate, discussing the roles that have been attributed to this molecule over time.

Keywords: lactic acid; brain metabolism; lactate transporters; blood-brain barrier; lactate receptors; exercise and lactate

1. Introduction

Carl Wilhelm Scheele, an 18th century Swedish chemist, also known for his role in the discovery of oxygen, purified many organic acids, among which lactic acid (LA).

About one hundred years later, in their study on the regulation of the blood supply to the brain, Roy and Sherrington reported that cerebral anemia, experimentally obtained by closing the carotid and vertebral arteries, was immediately accompanied by an acid reaction in the cortex; then, after reopening the cerebral vessels, the acidity gradually disappeared. The Authors attributed the observed effects to the formation of (ethylidene) lactic acid [1]. In the following years, it was proposed that LA increased during muscle exercise because of an oxygen debt, determined by the high energy requirement of contraction [2–4]. In glycolysis, indeed, glucose is broken down to two molecules of pyruvate; pyruvate then either enters the mitochondria, where it is further oxidized in the tricarboxylic acid (TCA) cycle, or is reversibly reduced by NADH(H^+)-dependent lactic dehydrogenase (LDH) to lactic acid, in order to regenerate NAD^+ for glycolysis. Shortage of oxygen, by slowing down mitochondrial oxidative

phosphorylation, and hence TCA cycle, causes an increase of lactate production. In order to limit cell acidification, both lactate and protons then exit cells.

By the end of the fifties, it was clear that hypoglycaemia, by limiting the supply to the brain of its preferred metabolic substrate (i.e., glucose), could reduce cell respiration/energy production and hence brain electric activity. It was also clear that, upon electrical stimulation, the metabolic activity of the brain could be increased and, as a consequence, more glucose and oxygen were taken from the blood, with a parallel increase of carbon dioxide and lactate released into the blood [5]. Moreover, electrical stimulation of brain tissue rendered it more susceptible to a fall in glucose level, and the same effect was noticed for the aerobic accumulation of LA [6]. These observations suggested that neuronal activity needs to be coupled to an efficient uptake of oxygen and metabolites from the circulation, and that the blood flow itself should be modulated locally in response to variations of neuronal activity [7]. In the following decades, the possibility emerged that cells in the brain itself, namely astrocytes, can transfer to neurons molecules, such as glucose and/or lactate, to sustain increased energy requirements, both in physiological and pathological conditions. Here, we'll discuss how ideas on the production and utilization in the brain of LA have changed over time from identification of lactate as a metabolic product of glucose metabolism to acknowledgement of its regulatory functions.

2. Exercise, Lactate Production in the Periphery and Fatigue

2.1. Exercise and Lactate Production

In muscle cells, during intense exercise, ATP is mainly generated from blood glucose and muscle glycogen to fuel glycolysis. This latter pathway is still considered the fastest, but produces less ATP compared to phosphagen systems and to the cellular respiration (oxidative phosphorylation, OXPHOS) [8]. ATP, the fuel used for contraction, is usually present in cells at a concentration of 8 mmol/kg of wet weight of muscle [9]. On the other hand, accumulation of pyruvate, not used for OXPHOS, should inhibit glycolysis because of shortage of NAD^+; to overcome this situation, in anaerobic conditions (hypoxia), a large part of pyruvate is reduced to lactate by lactate dehydrogenase enzyme, thus oxidizing $NADH(H^+)$ back to NAD^+. Noteworthy, during intense physical activity, lactic acid, that is a strong organic acid, mainly exists, like pyruvate, as an anion at the human body pH values. In other words, due to its pKa (3.86), lactic acid is physiologically deprotonated and exists as lactate and H^+ [10].

In addition, lactate can be also considered an aerobic metabolite, usable by skeletal muscles and by the heart when the oxygen availability is adequate, and it may contribute to acetyl-CoA formation [11]. A large part of muscle-derived lactate is transported to the liver where it is used to synthesize glucose through gluconeogenesis; glucose then enters the blood flow and comes to the muscles to be used as a metabolite in glycolysis (Cori's cycle). In the last decade, it has been discovered that adipocytes can also produce lactate under anaerobic circumstances. It has been suggested that, in order to counteract O_2 shortage, adipocytes switch to glycolytic metabolism, and to avoid a dramatic intracellular pH decrease, protons are removed from the cell thanks to proton-linked monocarboxylate transporters (MCTs; isoforms 1 and 4; see next section) [12]. The total amount of lactate produced by adipocytes depends on the fat mass: it is indeed higher in obese subjects than in normal-weight individuals.

A large part of lactate produced from active muscles is released and taken up by exercising and non-exercising muscles, heart and brain, to be oxidized and used as a fuel [13]. More than 30 years ago, Brooks introduced the concept of lactate shuttle through which lactate might be carried into mitochondria or peroxisomes, through MCTs, to be reoxidized, or even out into the bloodstream to be taken up from other organs which could use it as a fuel [14]. In sports medicine, the highest level of physical effort that the body can sustain without accumulating lactate and hydrogen ions (H^+) in blood and muscles is called anaerobic threshold or lactate threshold; this threshold also corresponds to the intensity/duration of physical activity that the energy metabolism supports without switching from aerobic to anaerobic. Lactate threshold provides a good fitness level and is considered

the best performance predictor. Intense training increases lactate threshold and, finally, increases performance [15].

Hypoxia can be induced by intense exercise as well as by altitude exposure (2300–5700 m); the latter induces skeletal muscle adaptations, such as transport of bicarbonate, hydrogen ions, and lactate, all of which are finely regulated. Oxygen shortage stimulates glycolysis and can increase pyruvate availability. This may be used for further oxidation in mitochondria or to increase lactate production. As a result of hypoxia, there is a higher amount of lactate production for a given workload. Muscular energy comes more from carbohydrates than fatty acids. Altitude-induced hypoxia leads to the lactate paradox, defined as "reduced blood lactate concentrations during submaximal and maximal exercise following acclimatization to high altitude" [16]. An interesting hypothesis is that altitude acclimatization may be due to a reduced "muscle recruitment" probably aimed at "protecting" cerebral oxygenation; in other words, the paradox of lactate, observed in chronic hypoxia, should be due to reduced muscle recruitment imposed by the central nervous system, that reduces the transport of O_2 to muscles and muscle contraction to protect itself from dangerous hypoxia [16,17].

2.2. Fatigue

A parallel interesting matter concerns "fatigue", a condition of rapid deterioration of contractile function, caused by intense exercise. Keeping ATP levels is essential in carrying out the physiological functions of the body. Muscle fatigue is possibly necessary to avoid that ATP range falls below critical or irreversible levels, and it is logical to assume a causal relationship between anaerobic metabolism and muscle fatigue [18]. The shift from aerobic to anaerobic metabolism is probably responsible for the sudden increase in lactate levels in blood and, even before, at the intracellular level, for metabolic acidosis [19]. Acidosis seems to impair muscle contraction, leading to fatigue and, ultimately, to cessation of exercise. The uncomfortable feelings associated with acidosis has long been associated with the pain (now commonly referred to as delayed onset muscle soreness, or DOMS) of the days after intense exercise. Lactate production through the glycolytic pathway is associated with hydrogen ions (H^+) production: in particular, two ions are released if glycolysis uses blood glucose, while one ion is released if glycogen is used [20]. Thus, lactate is not necessarily the source of the H^+ increase, which might indeed derive from glycolysis [21]. Historically, the accumulation of lactic acid in muscles has been suggested as the main cause of muscle fatigue. Lactate and H^+ ions are produced in muscles during intense exercise, and in humans, the intracellular lactate concentration may reach 30 mM or more. Nevertheless, the intracellular pH only decreases of approximately 0.5 units, due to the body buffering capacity. In these conditions, an increase of extracellular osmolarity should be observed, which causes a water movement out of the muscle fibers, and thereby an increase of the intracellular ionic strength. The overall result should be an inhibitory effect on force production [22,23]. On the other hand, acidification per se seems to have a low effect on the reduced fiber shortening velocity linked to fatigue. Lactic acid production plays indeed a metabolic buffering action rather than determining the onset of acidosis. As a matter of fact, the reaction catalyzed by lactate dehydrogenase uses two electrons and one proton from NADH and a second proton from the cytosol to reduce pyruvate to lactate [24]. In summary, the muscle lactate production is essential for the buffering effect as well as to regenerate NAD^+, at the same time serving to remove the extra pyruvate [9]. A further mechanism by which intracellular acidosis might induce fatigue is through inhibition of key enzymes of glycogenolysis and glycolysis, such as glycogen phosphorylase and phosphofructokinase, both of which are inhibited by low pH, at least in vitro [25].

Another system responsible for ATP synthesis in working muscles involves the phosphagens. During intense exercise, the main substrate used is creatine phosphate (CrP). The reaction catalyzed by creatine kinase uses protons (H^+), thus causing a faint alkalization of the muscle cell. The effect of this reaction is to counteract the slight acidosis induced by glycolysis and lactic acid formation [26]. Muscle contraction also causes an increase of phosphate (Pi) levels, which has a direct effect on myofibrils and/or an effect on the pathway of excitation/contraction of muscle cells [27].

Several studies suggest that an elevated muscle [H^+] as well as high [Pi] could impair muscle function by reducing (i) the transition of miofibrils to high cross-bridged states; (ii) the maximal shortening velocity; (iii) the glycolytic rate; (iv) cross-bridging activation, by competitively inhibiting Ca^{2+} binding to troponin C [4,28,29].

It is not yet clear which effect has the predominant role in fatigue; probably, all the mentioned factors are involved, and play a synergistic effect, thus leading to impaired contraction and muscle strength.

3. Lactate Uptake across the Blood-Brain Barrier and Monocarboxylate Carriers (MCTs)

3.1. Lactate Can Cross the Blood-Brain Barrier (BBB)

During the eighties, the idea that lactic acid (LA) was only a dead-end waste product of anaerobic metabolism began being challenged by many converging observations [4]. First of all, as mentioned, it was realized that LA can be shuttled both between and within the cells. The most relevant output of LA from cells was bound to sustained exercise. This process was defined by Brooks [14] as the "lactate shuttle". LA is indeed released from muscles during short-term exercise, and even from muscles at rest, during recovery from short-term exercise, and during long-lasting exercise. However, LA can be taken up from blood into either resting or slowly working muscles [4]. Actually, in the same muscle, glycolytic fibers can produce and release LA which can be, at least in part, taken up by surrounding oxidative fibers and again converted to pyruvate for further aerobic oxidization.

As we will discuss later, a relevant case of intercellular lactate shuttle should be that established among glial cells (especially astrocytes) and neurons, which could be considered one of the many aspects of the glial–neuronal metabolic coupling.

In addition to intercellular lactate shuttling, intra-cellular shuttling has been also suggested. In particular, muscular mitochondria were reported to contain a monocarboxylate transporter (MCT1, see below), able to allow LA entrance, as well as lactic dehydrogenase, which converts lactate back to pyruvate. This reaction also transfers electrons to NAD^+, thus also shuttling electrons from the cytosol to the mitochondrion [4,30,31].

A further indication that lactate is not only a waste product of anaerobic metabolism, produced in conditions of oxygen shortage, but instead a potentially useful fuel, came from the observation that the blood brain barrier (BBB) can be crossed by lactate. The brain is indeed isolated and protected from compounds circulating in the blood by a layer of brain capillary endothelial cells (BCECs) with peculiar properties [32]. BCECs are sealed together by tight junctions (TJs) which block para-cellular fluxes of molecules. Thus, in order to enter the brain, molecules have to be either soluble in plasma membrane lipids (and not actively extruded by ATP-dependent transporters: ABC transporters), or to be recognized by transporters, able to mediate trans-cellular crossing of the BCEC layer [32–35]. In addition, to ensure trans-cellular transport, a synergic cooperation between the two membranes of endothelial cells is required [36].

3.2. The Monocarboxylate Carriers (MCTs)

Lactate entrance into the brain is mediated by BBB transporters which belong to the monocarboxylate carrier (MCT) family, and also transport other monocarboxylic acids, such as pyruvate and ketone bodies. MCTs form a family of 14 transmembrane proteins [37,38], which belong to the solute carrier family (SLC16), and for many of which endogenous functions and substrates still await to be discovered. Actually, lactate transport across the cell plasma membranes of the body can use either sodium-coupled transporters (SMCTs) or proton-coupled monocarboxylate transporters (MCTs) [13]. In the brain, lactate transport involves MCTs. In particular, four carriers (MCTs 1–4) have been characterized which catalyze bidirectional, electroneutral 1:1 co-transport of protons and monocarboxylic acids [13,37–40]. All of them can transport lactate, with different affinities, the one with the highest affinity being MCT2 (Km: about 0.7 mM). MCT1 is expressed in a variety of human tissues,

including brain, and is involved in brain uptake of lactate across the BBB both in mice and humans [41]. MCT2, first reported to be expressed in astrocytes, has been then demonstrated to be primarily present in neurons, and, more precisely, in postsynaptic elements of glutamatergic synapses, where it has been suggested to provide increased supply of lactate "as energy fuel" during neuronal increased activity [39]. MCT3 is preferentially expressed in the basolateral membrane of the retinal pigment epithelium (RPE), and plays an important role in regulating pH and lactate concentration in the outer retina [42]. Finally, MCT4 (Km: about 35 mM) seems to be restricted to the plasma membrane of astrocytes, where it localizes to both perisynaptic processes and perivascular endfeet [39]. The different localization of the MCTs in the brain suggests that each species might be involved in different aspects of lactate metabolism, possibly involving differences in the preferential direction of inter-cellular lactate transport. The role of these transporters might change under different conditions. It has been reported, for example, that oxygen- and glucose-deprivation (OGD) increases cell death in cultured neurons, and up-regulates expression of MCT4 in astrocytes. If, however, cell cultures are exposed to MCT4- or MCT2-specific siRNAs, thus significantly reducing their expression, neuronal cell death increases, unless lactate is added to the culture medium before OGD [43]. Moreover, expression of MCTs has been found to be perturbed in some pathological conditions, such as epilepsy [41]. The MCTs have different Km for lactate, thus probably contributing to the specific tendency of each cell type in a tissue to transport lactate out of or into the cell. It is, however, to be pointed out that the proton gradient is also important [39].

Interestingly, membrane localization and activity of MCTs also require accessory proteins, such as neuroplastin, basigin and embigin [44–46], that chaperone their transport to the membrane as well as their function.

Recently, MCT1, MCT2, and MCT4 have been shown to be expressed also in the peripheral nervous system (PNS), with different cell- and domain-distribution: myelinating Schwann cells (SCs) express both MCT1 and MCT4, but in different compartments, while neurons of the dorsal root ganglion express MCT1. Interestingly, downregulation of MCT1 expression can increase myelinating activity in SCs and decrease neurofilament synthesis in neurons [47]. Moreover, the Authors found that lactate homeostasis participates in the regulation of SC myelinating program [47].

4. Glial Cell—Neurons Lactate Shuttle and Brain Energy Metabolism

4.1. Lactate Shuttling and Energy Metabolism

In the last two decades it has become increasingly clear that glial cells have more functions and are more dynamic than previously expected. Astrocytes, in particular, constitute with neurons a highly integrated complex in which each astrocyte contacts many neurons, and many astrocytes are coupled to each other through astroglial intercellular networks based on gap junction channels (GJ) [48–50], formed by connexins (C × 43 and C × 30) [51–53]. Importantly, astroglial networks are involved in several control functions, such as ionic homeostasis and control of cell volume; they seem to finely tune neuronal circuits, by delivering to neurons energy metabolites in neuronal activity-dependent manner [49], as also suggested by their distribution and proximity to excitatory synapses [54]. Moreover, C × 43 hemichannels have been reported to allow diffusion of gliotransmitters (for example, glutamate or ATP) [55,56], thus mediating signaling events which seem to be important also for functions as complex as memory consolidation [57], and recovery after ischemic injury [58]. Formation of an astrocytic network also allows generation of a glucose gradient, from the highest concentrations close to the BBB, where glucose uptake at astrocytic feet is maximal, to the lowest concentrations at the level of neuron-astrocyte contacts. Along this gradient, glucose can rapidly move toward the regions at which neuronal activity and hence metabolic demand are higher. Astrocytes are also able to store glucose as glycogen, and it has been proposed that this glycogen is metabolized and supports neuronal activity in conditions of glucose shortage, triggered by a variety of causes [59–63]. Interestingly, glycogen is located in myelinating Schwann cells in the mouse sciatic nerve which is

also able to release lactate, thus suggesting that glycogen plays a similar metabolic role in central and peripheral nervous system [64].

Finally, and most important, since many years ago, the existence of an astrocyte-neuron lactate shuttle (ANLS), which should be particularly active during excitatory neurotransmission, has been suggested [65]. In detail, the hypothesis moved from the observation that glutamate released from glutamatergic axon is taken up by both neurons, and astrocytes which surround the synapse. Glutamate uptake is mediated by a sodium-dependent carrier and triggers an increase of the Na^+/K^+-ATPase activity; by consuming ATP this process stimulates glycolysis, glucose utilization, and lactate production [65–68]. Then, lactate should be delivered to neurons through the MCTs present in both astrocytes and neurons. Finally, neurons should convert lactate to pyruvate and use it for synthesizing acetyl-CoA, and fueling the tricarboxylic acid cycle. At the same time, ATP is used in astrocytes to convert glutamate to glutamine which is released to neurons as well [69].

The central, and most important, point of the ANLS model is the neuronal use of astrocyte-derived lactate instead of glucose. Actually, even before the proposal of the lactate shuttle hypothesis, it had been demonstrated that brain slices in culture were able to maintain normal synaptic function using lactate as the only energy source [70], and that lactate, not glucose, was necessary to neurons during recovery from hypoxic conditions [71,72]. Moreover, as discussed by Schurr [73], the idea that brain tissue could oxidize lactate was even older and dates back to the thirties [74–78].

A further important aspect of the ANLS theory is the idea that lactate can come from glial cells [79–81]. There is some debate, however, on the origin of this lactate: as already mentioned, the original theory moved from glutamate recovery from the synapse [7,82]. An alternative proposal was that lactate derives from glycogen stored in astrocytes [83,84]. In contrast, it has also been suggested that it derives from slow oxidation of glutamate released from astrocytes in a calcium-dependent manner, and not from glutamate released during glutamatergic neuronal activation [85]. Further controversy concerns the real importance, under physiological conditions, of lactate released from astrocytes for neuronal metabolism [61,86]. It has been proposed, for example, that glycogenolysis' main function in astrocytes is to maintain a high concentration of glucose-6-phosphate, thus inhibiting hexokinase and sparing glucose; this latter could be thus diverted to neurons [62]. A fundamental question is which cell type (neurons or glial cells) uses more glucose? Under resting conditions, about one half of glucose seems to be taken up by neurons and one half by astrocytes. However, the energy required by astrocytes accounts for about 10%–15% of total energy needed in the brain, and that required by neurons for about 60% or more. This observation suggests that: (i) astrocytes use glucose mainly for anaerobic glycolysis; and (ii) neurons should use something else besides glucose [87]. Lactate might be the substrate produced in astrocytes and used in neurons, as shown at least in vitro [88–92]. Intriguingly, an opposite flux of metabolites (lactate and aspartate) from neurons to Muller glia has been reported in mouse retinas [93]. In addition, experimental evidence has been reported that, during neuronal activation, both neurons and astrocytes can oxidize glucose as well as lactate, and that considerable lactate production might be neuronal [94–100]. In agreement with the ANLS hypothesis, it has been found that exposure to metabolic stress induces the rapid release from neurons of tissue-type plasminogen activator (tPA). In this case tPA does not induce cleavage of plasminogen but, instead, AMPK activation and recruitment to the membrane of GLUT-1, in both astrocytes and endothelial cells. Uptake and metabolism of glucose is followed by production and release of lactate, which is then captured by neurons through the MCT-2 [101]. Interestingly, astrocyte-neuron lactate transport has also been suggested to be required for long-term memory formation (see below); in particular, it seems that, during the most intense cognitive activities in the hippocampus, astrocytic glycogenolysis is stimulated and provides lactate, which is then transferred to neurons as an energy surplus when glucose is not sufficient [102,103]. In agreement with this idea, it has been recently reported that, in response to local neuronal activation, lactate is released from astrocytes through a K^+-stimulated anion channel [104].

On the other hand, other researchers reported that, during neuronal activity in hippocampal slices, glucose is the effective energy substrate for both neurons and astrocytes [105,106]. The mentioned controversies could be at least partially solved if lactate is not only a metabolic substrate but also a signaling molecule (see below).

4.2. Glucose Sensing

A particularly interesting topic concerns the ability of the brain to "sense" glucose concentration. This ability primarily involves the hypothalamus, the part of the brain able to organize adaptive responses by modulating different functions, among which hormone production, which in turn regulates food intake and peripheral organ activities. In contrast with other brain regions, hypothalamus (and in particular the arcuate nucleus, ARC) is not completely separated from circulation by the BBB and spaces permeable to ions and nutrients are present, close to the nutrient-sensitive neurons [50,107]. Central glucose sensing, in turn, controls neuronal signaling cascades which regulate peripheral hormone-dependent glucose uptake and metabolism [108].

Glucose-sensitive neurons were discovered about 50 years ago [109,110] and their mechanisms of action have been further characterized by ex vivo electrophysiological studies [50]. Recently, the mechanisms underlying glucose sensitivity have been better clarified with the discovery that astrocytes, and specifically connexins, are also involved [111]. Although the main cerebral forms of glucose transporters are the high-affinity ones (GLUT1 and GLUT3), similarly to pancreatic beta cells, hypothalamus glucose sensitivity relies on GLUT2 glucose transporter and on the ability of hexokinase IV (glucokinase, GK) to phosphorylate glucose, without being inhibited by its end-product, glucose-6-phosphate. GK is expressed in selected neuronal populations of hypothalamus [108,112], and, in addition, it has been reported to form a mitochondrial complex with proteins involved in apoptosis control [113]. On the other hand, GLUT2 is mainly present in hypothalamic astrocytes [114,115], thus suggesting that these cells have an important role in sensing glucose concentration. Interestingly, it has been reported that glucose-sensing neurons can also be activated by lactate [116,117], and one possible view is that they sense, at least in part, not directly glucose, but lactate released by astrocytes [50].

4.3. Role of Extracellular Vesicles (EVs) in Cell-to-Cell Communications in the Brain

Finally, a short mention should be made on the molecule exchange among brain cells, which involves extracellular vesicles (EVs), either ectosomes or exosomes. Both neurons [118] and astrocytes [119] in culture can release into the medium extracellular vesicles (EVs) which contain, for example, angiogenic factors [118,119], molecular chaperones [120], and synapsin I [121]. This mode of communication is fundamental for tumor cells, including brain cancer cells, for invasion and migration [122–124], but it could also have an important role in normal brain cells. Brain cells have indeed the ability to grow branched cellular processes which explore the environment and establish several contacts with other cell types. Interestingly, in vitro studies showed that synaptic activity can potentiate release of exosomes from neurons [125], and that exosomes can be involved in synaptic plasticity and long-term memory [126]. EVs are also released from oligodendrocytes and it has been reported that these vesicles can contain molecules of metabolic interest, among which lactate [127–129].

5. Lactate as a Substrate during Exercise and in Memory Processes

A growing body of evidence suggests that mild, but regular, physical activity can be beneficial to neuronal survival and plasticity. This observation is especially important in older people. It is indeed clear that increasing age is associated with decreasing cognitive functions, and increasing risk of dementia. It has been reported for example that the number of deaths from Alzheimer's disease (AD) increased by 68% from 2000 to 2010, in spite of a drastic reduction of mortality from cardiovascular disease and stroke [130]. In general, brain volume decreases with aging. Cognitive decline has been attributed to changes in both neuronal and non-neuronal populations within the CNS, including deterioration of the blood–brain barrier (BBB) [131]. Physical activity seems to ameliorate cognition

processes, and counteract brain aging [132,133]. The cellular events responsible for these effects have been, however, only partially characterized.

As expected, during exercise, brain blood flow increases, and this elevation is due to an increase in cardiac output, but probably also to changes in brain metabolism [134]. For example, brain glycogen stored in astrocytes decreases during prolonged exercise with hypoglycemia, and this effect has been attributed to activation of the transduction pathways triggered by noradrenaline and serotonin [135–137]. By using an acute intense exercise model of swimming in rats, Matsui et al. [63] also showed that glycogen decreases also in the absence of hypoglycemia, and that its decrease associates with increased lactate in hippocampus, cerebellum, cortex and brain stem [63]. Since glycogen is essentially stored in astrocytes, it is likely that the increase of lactate is due to astrocytic activity. As mentioned, it has been suggested that astrocyte-neuron lactate transport is also important for memory formation [102,103,138]. Could lactate be the link between exercise and improvement of cognitive functions? One of the factors that link exercise and memory seems to be brain-derived neurotrophic factor (BDNF) [139–141]. Animal studies have shown that this neurotrophin is essential for long-term potentiation (LTP) and neuroplasticity [142–144]. Moreover, pharmacological blockade of BDNF expression hampers the ability to acquire and retain novel spatial information in rats exposed to exercise [145]. Notably, human studies have confirmed that circulating levels of BDNF are transiently increased with exercise [146,147]. Mild exercise can also stimulate hippocampal neurogenesis [148]. However, the biochemical mechanisms underlying the link between exercise-related peripheral BDNF increases and memory improvements in humans remain to be clarified. Two other growth factors are probably involved in the effects of exercise on neuroplasticity: insulin-like growth factor 1 (IGF-1) [149–151] and vascular endothelial growth factor (VEGF) [141]. VEGF could have a direct effect on BBB function and, in turn, on oxygen and glucose uptake to the brain, while, interestingly, both IGF-1 and BDNF seem to stimulate neurogenesis, through a signaling cascade that includes calmodulin-dependent protein kinase II (CAMK-II), an enzyme likely involved in long term potentiation [152,153]. CAMK-II is regulated by calcium ions and calmodulin (CaM). In neurons, CaM also interacts with small IQ domain proteins (SNIQ), such as neuromodulin, neurogranin, and PEP-19 [154–157]. SNIQ have the potential to modulate the amount/localization of calmodulin which can interact with, and activate CAMK-II. This raises the interesting possibility that IQ domain proteins might provide at least some of the still unknown regulatory links between calcium metabolism in the brain and cognitive functions, in both normal and pathological conditions. For example, CAMK-II might phosphorylate RNA-binding proteins which are part of pre-localized ribonucleoparticles (RNPs); phosphorylation of these proteins should then allow translation of specific mRNAs only at the level of activated synapses, thus participating in their potentiation [158]. Notably, at least one of the small IQ domain-containing proteins, PEP19, has been reported to be able to bind mRNA, in alternative to calmodulin [159].

Coming back to lactate, this compound, possibly supplied to neurons from astrocytic glycogenolysis, seems to be an important substrate for neuronal metabolism and LTP maintenance [141]. In addition, during exercise, lactate originating from muscle metabolism can also be shuttled across the BBB through monocarboxylate transporters (MCTs), to be used by brain cells [39,134,160,161]. Interestingly, increases in peripheral blood lactate levels have recently been associated with increased circulating BDNF [162,163]. The biochemical association between the neurotrophin and lactate production has not yet been clearly understood. However, this observation, together with the putative role of lactate in neuron metabolism under conditions of special energy demand, suggests that lactate can indeed represent the link between exercise and exercise-dependent improvement of cognitive functions. Actually, memory formation and consolidation require energy, especially in conditions of arousal and/or stress, and, as mentioned, this energy seems to mostly derive from astrocytic glycogenolysis. In the hippocampus, exercise leads to a burst of extracellular lactate which remains at high levels for at least 50 min and is completely blocked by inhibition of glycogenolysis, which also blocks long-term memory [102]. An important role in memory processes is played by glutamate, and

active uptake of glutamate by astrocytes, as already mentioned, is the basis of the astrocyte-neuron shuttle [138].

Moreover, lactate has been recently reported to potentiate NMDA glutamate receptor-mediated currents, which play a central role in neuronal plasticity and memory processes; in doing that, lactate activates a cascade of molecular events which ends up with stimulation of the expression of synaptic plasticity-related genes, such as Arc, c-Fos, and Zif268 [164].

6. Lactate as a Signaling Molecule in the Brain

As discussed above, lactate is probably a molecule with an important metabolic impact in the brain. However, a further role is emerging [165,166]: lactate can indeed also bind a receptor of the G protein coupled receptor (GPRs) family: GPR81, also known as HCA1 (hydroxycarboxylic acid 1) or HCAR1 (hydroxycarboxylic acid receptor 1). This receptor is connected to a G protein (Gi) that inhibits adenylate cyclase, thus causing a decrease of the second messenger cyclic AMP (cAMP). The mRNA encoding GPR81 and the protein itself localize to hippocampus, neocortex and cerebellum. The protein is concentrated in the principal neurons (for example, the pyramidal neurons in the hippocampus), but also in the interneurons of many brain areas [167]. By electron microscopy with immunogold, the highest concentration of the receptor was found in the somatodendritic compartment, mainly on the post-synaptic dendritic spines of excitatory synapses [166]. Immunoreactivity was also found on the brain capillary endothelial cells (BBB) and on the perivascular and perisynaptic astrocytic processes. Localization of GPR81 thus suggests that lactate can have at the same time a metabolic role and a regulatory role in the control of blood flow and synaptic function, acting as an intercellular messenger [168]. Moreover, transduction of a signal which decreases cAMP could represent a feedback mechanism, opposing the effect of catecholamines, which induce glycogen breakdown and production of the lactate itself [169,170]. Possibly, signaling through GPR81 (which requires high concentrations of lactate) may have a special role under stress conditions, such as those induced by ischemia or seizures. Actually, it seems that lactate can contribute to signaling pathways in other ways: for example, lactate released from astrocytes attenuates transporter-mediated uptake of prostaglandin E2, thus increasing its external accumulation and vasodilation [171]. However, vasodilation is useful in hypoxia or, in general, in conditions of energy deficiency, which are the conditions of high lactate production and release. Thanks to vasodilation a higher amount of oxygen and glucose can reach the brain.

Interestingly, a different, putatively excitatory G-protein coupled receptor for lactate, which causes an increase of cAMP, has been described in the Locus Coeruleus, but its significance is still under study [170].

Lactate has also been reported to activate the expression of NDRG3 protein. During hypoxia, low oxygen concentration and high lactate levels induce NDRG3 protein that, in turn, stimulates the Raf-ERK pathway to promote angiogenesis and cell growth [172].

Moreover, Rinholm and colleagues showed that low glucose medium conditions reduce number and myelination activity of cultured oligodendrocytes, and that the addition of lactate can rescue both development and myelinating capacity of the cells [173].

Taken together, these observations indicate that lactate plays a very complex role in brain metabolism and function. This role is possibly different in normal and stress/pathological conditions. Thus a final central question is: are elevated concentrations of lactate in the brain the cause or the consequence of stress/pathological conditions?

7. Brain Glucose Metabolism and Elevated Lactate in Pathological Conditions

In many acute as well as chronic pathological conditions, brain glucose metabolism is altered. For example, diabetic patients undergo frequent episodes of hypoglycemia, due to the concomitant effects of the therapy itself and the inability of the organism to elicit the physiological responses to a low concentration of circulating glucose [174]. In these patients, as well as in nondiabetic people, lactate infusions can reduce the response to epinephrine, thus reducing the risk of hypoglycemia, while

causing brain lactate uptake [161,174–177]. Similar observations have been done in rats [178]. Now, as discussed above, beside the use of lactate coming from the circulation as a substrate in the place of glucose, an additional explanation for these observations could be that lactate, by binding to its GPR81 receptor, induces a decrease of cAMP, thus counteracting the effects of epinephrine.

In traumatic brain injury (TBI), an increase of brain glycolysis, partially relying on brain glycogen, is observed immediately after injury, in parallel with a decrement in cerebral oxygen consumption rate [179]. In some patients, an elevation of the pentose phosphate pathway was also observed [180]. In the days following TBI, however, glucose uptake and its use rate decrease. At the same time, many patients show cerebral net lactate uptake [181]. A direct use of lactate in patients with TBI was actually demonstrated by a combination of 13C-labelled microdialysis and high-resolution nuclear magnetic resonance [182,183]. For this reason, lactate is beginning to be considered a good substrate for vascular infusion of patients in the days after TBI; lactate indeed should enter the mitochondria to be oxidized to pyruvate and pyruvate could be then used for fueling the TCA cycle. Alternatively, lactate could be oxidized to pyruvate in the cytoplasm and pyruvate could enter the mitochondria. In both cases, lactate should serve as a "glucose sparing" substrate [179,184].

Notably, on the other hand, some Authors have emphasized the uncoupling of metabolism between neurons and astrocytes after TBI, and the production of an excessive amount of lactate, which can be toxic, in the injured brain ("lactate storm") [185].

In the case of ischemic insults, many in vitro studies suggest that lactate is the major energy substrate for surviving neurons, and it has been reported that lactate can protect neurons from glutamate-induced neurotoxicity [39,186]. Intracerebroventricular as well as intravenous administration of lactate after transient middle cerebral artery occlusion attenuate lesion size and improve neurologic outcome. These effects can be partially explained by the use of lactate as a metabolic substrate and partially by its binding to GPR81, and activation of a signal transduction pathway in neurons [187,188].

Disturbances of glucose metabolism have also been recognized in the pathogenesis of depression. By studying in a model of prenatal stressed rats (an animal model of depression), the expression of different enzymes involved in glycolysis, pentose phosphate pathway, and TCA cycle, data have been collected which suggest that glycolysis is increased and TCA cycle decreased in the brain of prenatal stressed animals, with a parallel increase of lactate [189].

A significant increase in de novo synthesis of lactate (and glutamine) was also observed in the brain of rats which underwent experimental bile-duct ligation to simulate liver fibrosis and necrosis; these rats are a good animal model of chronic liver disease, as they indeed develop brain edema, and minimal hepatic encephalopathy. On the basis of these results, the Authors suggest that inhibiting lactate synthesis could be a target in the treatment of hepatic encephalopathy [190,191].

The levels of lactate were reported to be high in the cerebrospinal fluid (CSF) of patients with Alzheimer's Diseases (AD) [192]. Now, β-site APP-cleaving enzyme (BACE1), one of the two enzymes which cleave the amyloid precursor protein (APP) to give beta peptides, has an acidic optimal pH; because of this consideration, the levels of beta peptides have been studied in cultured neuroblastoma cells, treated with lactate. According to these studies, high levels of lactate could be a risk factor in AD amyloidogenesis because of aberrant APP processing leading to increased generation of amyloid peptides and APP aggregates; however, the lactate effect does not seem to be due to pH modifications, but to increased levels of ER chaperones Grp78 and Grp94, which leads to aggresome formation [193].

On the other hand, regular physical activity and exercise are protective against many pathologies, such as cardiovascular diseases, and dementia, including AD [130]. As we have discussed, exercise causes an elevation of circulating lactate and this lactate can reach the brain, by crossing the BBB. Notably, similarly good effects of exercise have been reported in Parkinson disease [194] and Huntington's Disease [195], as well as in multiple sclerosis [196].

As a final note, high levels of serum lactate have been reported to be non-invasive biomarkers of malignancy for brain tumors [197].

8. Conclusions

In conclusion, the idea that lactate is a waste product of the metabolism and a main cause of fatigue, should no longer be considered as an indisputable truth. This molecule, instead, can be shuttled among cells and, inside the cells, among different organelles, thanks to specific monocarboxylate carriers, and seems to be a fuel for many cells, including neurons, in conditions of oxygen shortage.

Most importantly, it can behave as a signal, eliciting in the cells' responses which counteract stress, allowing at least an attempt of adaptive responses. Interestingly, exercise, which promotes lactate production, has been recently reported to have a positive effect in many physiological as well as pathological conditions, including brain aging and neurodegenerative diseases.

Although more research is required to better understand the real function of lactate produced during exercise, what we have been knowing up to now is exciting and suggests that this molecule could somehow underlie the ancient observation that a healthy (and well exercised) body hosts a healthy mind.

Acknowledgments: This work was supported by University of Palermo (Università degli Studi di Palermo, Palermo, Italy).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Roy, C.S.; Sherrington, C.S. On the Regulation of the Blood-supply of the Brain. *J. Physiol.* **1890**, *11*, 85–158. [PubMed]
2. Fletcher, W.M.; Hopkins, F.G. Lactic acid in amphibian muscle. *J. Physiol.* **1907**, *35*, 247–309. [PubMed]
3. Hill, A.V.; Long, C.N.H.; Lupton, H. Muscular exercise, lactic acid, and the supply and utilization of oxygen. Part VI. The oxygen debt and the end of exercise. *Proc. R. Soc. Lond. B Biol. Sci.* **1924**, *97*, 127–137.
4. Gladden, L.B. Lactate metabolism: A new paradigm for the third millennium. *J. Physiol.* **2004**, *558*, 5–30. [PubMed]
5. McIlwain, H. Metabolic Response in vitro to Electrical Stimulation of Sections of Mammalian Brain. *Biochem. J.* **1951**, *49*, 382–393. [PubMed]
6. McIlwain, H. Glucose Level, Metabolism, and Response to Electrical Impulses in Cerebral Tissues from Man and Laboratory Animals. *Biochem. J.* **1953**, *55*, 618–624. [PubMed]
7. Magistretti, P.J. Neuron–glia metabolic coupling and plasticity. *J. Exp. Biol.* **2006**, *209*, 2304–2311. [PubMed]
8. Greenhaff, P.L.; Nevill, M.E.; Soderlund, K.; Bodin, K.; Boobis, L.H.; Williams, C.; Hultman, E. The metabolic responses of human type I and II muscle fibres during maximal treadmill sprinting. *J. Physiol.* **1994**, *478*, 149–155. [PubMed]
9. Baker, J.S.; McCormick, M.C.; Robergs, R.A. Interaction among skeletal muscle metabolic energy systems during intense exercise. *J. Nutr. Metab.* **2010**, *2010*. [CrossRef]
10. Crisp, A.H.; Verlengia, R.; Rocha, G.L.; da Mota, G.R.; Pellegrinotti, I.L.; Lopes, R.L. Lactate and monocarboxylate transporters (MCTS): A review of cellular aspects. *J. Exerc. Physiol. Online* **2015**, *18*, 1–13.
11. Chatham, J.C. Lactate—The forgotten fuel! *J. Physiol.* **2002**, *542*. [CrossRef]
12. Pérez de Heredia, F.; Wood, I.S.; Trayhurn, P. Hypoxia stimulates lactate release and modulates monocarboxylate transporter (MCT1, MCT2, and MCT4) expression in human adipocytes. *Pflugers Arch.* **2010**, *459*, 509–518. [PubMed]
13. Adeva-Andany, M.; López-Ojén, M.; Funcasta-Calderón, R.; Ameneiros-Rodríguez, E.; Donapetry-García, C.; Vila-Altesor, M.; Rodríguez-Seijas, J. Comprehensive review on lactate metabolism in human health. *Mitochondrion* **2014**, *17*, 76–100. [PubMed]
14. Brooks, G.A. The lactate shuttle during exercise and recovery. *Med. Sci. Sports Exerc.* **1986**, *18*, 360–368. [PubMed]
15. Facey, A.; Irving, R.; Dilworth, L. Overview of Lactate Metabolism and the Implications for Athletes. *Am. J. Sports Sci. Med.* **2013**, *1*, 42–46.

16. Noakes, T.D. Evidence that reduced skeletal muscle recruitment explains the lactate paradox during exercise at high altitude. *J. Appl. Physiol.* **2009**, *106*, 737–738. [PubMed]
17. Amann, M.; Romer, L.M.; Subudhi, A.W.; Pegelow, D.F.; Dempsey, J.A. Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J. Physiol.* **2007**, *581*, 389–403. [PubMed]
18. Vollestad, N.K.; Sejersted, O.M. Biochemical correlates of fatigue. A brief review. *Eur. J. Appl. Physiol. Occup. Physiol.* **1988**, *57*, 336–347. [PubMed]
19. Posterino, G.S.; Dutka, T.; Lamb, G.D. L(+)-lactate does not affect twitch and tetanic responses in mechanically skinned mammalian muscle fibres. *Pflügers Arch.* **2001**, *442*, 197–203. [PubMed]
20. Hall, M.M.; Rajasekaran, S.; Thomsen, T.W.; Peterson, A.R. Lactate: Friend or Foe. *PM&R* **2016**, *8*, S8–S15.
21. Robergs, R.A.; Ghiasvand, F.; Parker, D. Biochemistry of exercise induced metabolic acidosis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2004**, *287*, R502–R516. [PubMed]
22. Lamb, G.D.; Stephenson, D.G.; Stienen, G.J. Effects of osmolality and ionic strength on the mechanism of Ca2 release in skinned skeletal muscle fibres of the toad. *J. Physiol.* **1993**, *464*, 629–648. [PubMed]
23. Allen, D.G.; Lamb, G.D.; Westerbland, H. Skeletal Muscle Fatigue: Cellular Mechanisms. *Physiol. Rev.* **2008**, *88*, 287–332. [PubMed]
24. Bangsbo, J.; Madsen, K.; Kiens, B.; Richter, E.A. Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J. Physiol.* **1996**, *495*, 587–596. [PubMed]
25. Bruton, J.D.; Lännergren, J.; Westerblad, H. Effects of CO_2-induced acidification on the fatigue resistance of single mouse muscle fibers at 28 degrees C. *J. Appl. Physiol.* **1998**, *85*, 478–483. [PubMed]
26. Norman, B.; Sabina, R.L.; Jansson, E. Regulation of skeletal muscle ATP catabolism by AMPD1 genotype during sprint exercise in asymptomatic subjects. *J. Appl. Physiol.* **2001**, *91*, 258–264. [PubMed]
27. Allen, D.G.; Trajanovska, S. The multiple roles of phosphate in muscle fatigue. *Front. Physiol.* **2012**, *3*, 1–8.
28. Fitts, R.H. Mechanisms of muscular fatigue. In *Principles of Exercise Biochemistry*, 3rd ed.; Poortmans, J.R., Ed.; Karger: Basel, Switzerland, 2003; pp. 279–300.
29. Westerblad, H.; Allen, D.G.; Lännergren, J. Muscle fatigue: Lactic acid or inorganic phosphate the major cause? *News Physiol. Sci.* **2002**, *17*, 17–21. [PubMed]
30. Dubouchaud, H.; Butterfield, G.E.; Wolfel, E.E.; Bergman, B.C.; Brooks, G.A. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am. J. Physiol.* **2000**, *278*, E571–E579.
31. Brooks, G.A. Cell-cell and intracellular lactate shuttles. *J. Physiol.* **2009**, *587*, 5591–5600. [PubMed]
32. Abbott, N.J. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *J. Inherit. Metab. Dis.* **2013**, *36*, 437–449. [PubMed]
33. Smith, Q.R. Transport of glutamate and other amino acids at the Blood-Brain Barrier. *J. Nutr.* **2000**, *130*, 1016S–1022S. [PubMed]
34. Huber, J.D.; Egleton, R.D.; Davis, T.P. Molecular physiology and pathophysiology of tight junctions in the bloodbrain barrier. *Trends Neurosci.* **2001**, *24*, 719–725. [PubMed]
35. Pardridge, W.M. Drug transport across the blood-brain barrier. *J. Cereb. Blood Flow Metab.* **2012**, *32*, 1959–1972. [PubMed]
36. Hawkins, R.A.; Viña, J.R.; Mokashi, A.; Peterson, D.R.; O'Kane, R.; Simpson, I.A.; Dejoseph, M.R.; Rasgado-Flores, H. Synergism between the two membranes of the blood-brain barrier: Glucose and amino acid transport. *Am. J. Neurosci. Res.* **2013**, *1*, 1–25.
37. Pérez-Escuredo, J.; Van Hée, V.F.; Sboarina, M.; Falces, J.; Payen, V.L.; Pellerin, L.; Sonveaux, P. Monocarboxylate transporters in the brain and in cancer. *Biochim. Biophys. Acta* **2016**, *1863*, 2481–2497. [PubMed]
38. Jones, R.S.; Morris, M.E. Monocarboxylate Transporters: Therapeutic targets and prognostic factors in disease. *Clin. Pharmacol. Ther.* **2016**. [CrossRef]
39. Bergersen, L.H. Is lactate food for neurons? Comparison of monocarboxylate transporter subtypes in brain and muscle. *Neuroscience* **2007**, *145*, 11–19. [PubMed]
40. Halestrap, A.P. The monocarboxylate transporter family-structure and functional characterization. *IUBMB Life* **2012**, *64*, 1–9. [PubMed]
41. Lauritzen, K.H.; Eid, T.; Bergersen, L.H. Monocarboxylate transporters in temporal lobe epilepsy: Roles of lactate and ketogenic diet. *Brain Struct. Funct.* **2013**, *220*, 1–12. [PubMed]

42. Gallagher-Colombo, S.; Maminishkis, A.; Tate, S.; Grunwald, G.B.; Philp, N.J. Modulation of MCT3 expression during wound healing of the retinal pigment epithelium. *Investig. Ophthalmol. Vis. Sci.* **2010**, *51*, 5343–5350.
43. Gao, C.; Zhou, L.; Zhu, W.; Wang, H.; Wang, R.; He, Y.; Li, Z. Monocarboxylate transporter-dependent mechanism confers resistance to oxygen- and glucose-deprivation injury in astrocyte-neuron co-cultures. *Neurosci. Lett.* **2015**, *594*, 99–104. [PubMed]
44. Wilson, M.C.; Meredith, D.; Fox, J.E.; Manoharan, C.; Davies, A.J.; Halestrap, A.P. Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: The ancillary protein for the insensitive MCT2 is EMBIGIN (gp70). *J. Biol. Chem.* **2005**, *280*, 27213–27221. [PubMed]
45. Ovens, M.J.; Manoharan, C.; Wilson, M.C.; Murray, C.M.; Halestrap, A.P. The inhibition of monocarboxylate transporter 2 (MCT2) by AR-C155858 is modulated by the associated ancillary protein. *Biochem. J.* **2010**, *431*, 217–225. [PubMed]
46. Wilson, M.C.; Kraus, M.; Marzban, H.; Sarna, J.R.; Wang, Y.; Hawkes, R.; Halestrap, A.P.; Beesley, P.W. The neuroplastin adhesion molecules are accessory proteins that chaperone the monocarboxylate transporter MCT2 to the neuronal cell surface. *PLoS ONE* **2013**, *8*, e78654.
47. Domènech-Estévez, E.; Baloui, H.; Repond, C.; Rosafio, K.; Médard, J.J.; Tricaud, N.; Pellerin, L.; Chrast, R. Distribution of monocarboxylate transporters in the peripheral nervous system suggests putative roles in lactate shuttling and myelination. *J. Neurosci.* **2015**, *35*, 4151–4156. [PubMed]
48. Giaume, C.; Koulakoff, A.; Roux, L.; Holcman, D.; Rouach, N. Astroglial networks: A step further in neuroglial and gliovascular interactions. *Nat. Rev. Neurosci.* **2010**, *11*, 87–99. [PubMed]
49. Pannasch, U.; Rouach, N. Emerging role for astroglial networks in information processing: From synapse to behavior. *Trends Neurosci.* **2013**, *36*, 405–417. [PubMed]
50. Leloup, C.; Allard, C.; Carneiro, L.; Fioramonti, X.; Collins, S.; Pénicaud, L. Glucose and hypothalamic astrocytes: More than a fueling role? *Neuroscience* **2016**, *323*, 110–120. [PubMed]
51. Lauf, U.; Giepmans, B.N.; Lopez, P.; Braconnot, S.; Chen, S.C.; Falk, M.M. Dynamic trafficking and delivery of connexons to the plasma membrane and accretion to gap junctions in living cells. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10446–10451. [PubMed]
52. Moore, K.B.; O'Brien, J. Connexins in neurons and glia: Targets for intervention in disease and injury. *Neural Regen. Res.* **2015**, *10*, 1013–1017. [PubMed]
53. Bosone, C.; Andreu, A.; Echevarria, D. GAP junctional communication in brain secondary organizers. *Dev. Growth Differ.* **2016**, *58*, 446–455. [PubMed]
54. Genoud, C.; Houades, V.; Kraftsik, R.; Welker, E.; Giaume, C. Proximity of excitatory synapses and astroglial gap junctions in layer IV of the mouse barrel cortex. *Neuroscience* **2015**, *291*, 241–249. [PubMed]
55. Ye, Z.C.; Wyeth, M.S.; Baltan-Tekkok, S.; Ransom, B.R. Functional hemichannels in astrocytes: A novel mechanism of glutamate release. *J. Neurosci.* **2003**, *23*, 3588–3596. [PubMed]
56. Stout, C.E.; Costantin, J.L.; Naus, C.C.; Charles, A.C. Intercellular calcium signaling in astrocytes via ATP release through connexinhemichannels. *J. Biol. Chem.* **2002**, *277*, 10482–10488. [PubMed]
57. Stehberg, J.; Moraga-Amaro, R.; Salazar, C.; Becerra, A.; Echeverría, C.; Orellana, J.A.; Bultynck, G.; Ponsaerts, R.; Leybaert, L.; Simon, F.; et al. Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB J.* **2012**, *26*, 3649–3657. [PubMed]
58. Li, X.; Zhao, H.; Tan, X.; Kostrzewa, R.M.; Du, G.; Chen, Y.; Zhu, J.; Miao, Z.; Yu, H.; Kong, J.; et al. Inhibition of connexion43 improves functional recovery after ischemic brain injury in neonatal rats. *Glia* **2015**, *63*, 1553–1567. [PubMed]
59. Matsui, T.; Soya, S.; Okamoto, M.; Ichitani, Y.; Kawanaka, K.; Soya, H. Brain glycogen decreases during prolonged exercise. *J. Physiol.* **2011**, *589*, 3383–3393. [PubMed]
60. Chambers, T.W.; Daly, T.P.; Hockley, A.; Brown, A.M. Contribution of glycogen in supporting axon conduction in the peripheral and central nervous systems: The role of lactate. *Front. Neurosci.* **2014**, *8*, 1–6.
61. Dienel, G.A. The metabolic trinity, glucose-glycogen-lactate, links astrocytes and neurons in brain energetics, signaling, memory, and gene expression. *Neurosci. Lett.* **2015**. [CrossRef]
62. Dienel, G.A.; Cruz, N.F. Contributions of glycogen to astrocytic energetics during brain activation. *Metab. Brain Dis.* **2015**, *30*, 281–298. [PubMed]

63. Matsui, T.; Soya, S.; Okamoto, M.; Ichitani, Y.; Kawanaka, K.; Soya, H. Brain Glycogen Decreases during Intense Exercise without Hypoglycemia: The Possible Involvement of Serotonin. *Neurochem. Res.* **2015**, *40*, 1333–1340. [PubMed]
64. Evans, R.D.; Brown, A.M.; Ransom, B.R. Glycogen function in adult central and peripheral nerves. *J. Neurosci. Res.* **2013**, *91*, 1044–1049. [PubMed]
65. Pellerin, L.; Magistretti, P.J. Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10625–10629. [CrossRef] [PubMed]
66. Debernardi, R.; Magistretti, P.J.; Pellerin, L. Trans-inhibition of glutamate transport prevents excitatory amino acid-induced glycolysis in astrocytes. *Brain Res.* **1999**, *850*, 39–46. [CrossRef]
67. Voutsinos-Porche, B.; Bonvento, G.; Tanaka, K.; Steiner, P.; Welker, E.; Chatton, J.Y.; Magistretti, P.J.; Pellerin, L. Glial glutamate transporters mediate a functional metabolic crosstalk between neurons and astrocytes in the mouse developing cortex. *Neuron* **2003**, *37*, 275–286. [CrossRef]
68. Chatton, J.Y.; Magistretti, P.J.; Barros, L.F. Sodium signaling and astrocyte energy metabolism. *Glia* **2016**. [CrossRef] [PubMed]
69. Magistretti, P.J.; Pellerin, L. Astrocytes Couple Synaptic Activity to Glucose Utilization in the Brain. *News Physiol. Sci.* **1999**, *14*, 177–182. [PubMed]
70. Schurr, A.; West, C.A.; Rigor, B.M. Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* **1988**, *240*, 1326–1328. [CrossRef] [PubMed]
71. Schurr, A.; Payne, R.S.; Miller, J.J.; Rigor, B.M. Brain lactate, not glucose, fuels the recovery of synaptic function from hypoxia upon reoxygenation: An in vitro study. *Brain Res.* **1997**, *744*, 105–111. [CrossRef]
72. Schurr, A.; Payne, R.S.; Miller, J.J.; Rigor, B.M. Glia are the main source of lactate utilized by neurons for recovery of function posthypoxia. *Brain Res.* **1997**, *774*, 221–224. [CrossRef]
73. Schurr, A. Cerebral glycolysis: A century of persistent misunderstanding and misconception. *Front. Neurosci.* **2014**, *8*. [CrossRef] [PubMed]
74. Ashford, C.A.; Holmes, E.G. Contributions to the study of brain metabolism: Role of phosphates in lactic acid production. *Biochem. J.* **1929**, *23*, 748–759. [CrossRef] [PubMed]
75. Holmes, E.G. Oxidations in central and peripheral nervous tissue. *Biochem. J.* **1930**, *24*, 914–925. [CrossRef]
76. Holmes, E.G.; Ashford, C.A. Lactic acid oxidation in brain with reference to the "Meyerhof cycle". *Biochem. J.* **1930**, *24*, 1119–1127. [CrossRef] [PubMed]
77. Ashford, C.A.; Holmes, E.G. Further observations on the oxidation of lactic acid by brain tissue. *Biochem. J.* **1931**, *25*, 2028–2049. [CrossRef] [PubMed]
78. Quastel, J.H.; Wheatley, A.H. Oxidations by the brain. *Biochem. J.* **1932**, *26*, 725–744. [CrossRef] [PubMed]
79. Wender, R.; Brown, A.M.; Fern, R.; Swanson, R.A.; Farrell, K.; Ransom, B.R. Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J. Neurosci.* **2000**, *20*, 6804–6810. [PubMed]
80. Brown, A.M.; Wender, R.; Ransom, B.R. Metabolic substrates other than glucose support axon function in central white matter. *J. Neurosci. Res.* **2001**, *66*, 839–843. [CrossRef] [PubMed]
81. Pellerin, L. Lactate as a pivotal element in neuron-glia metabolic cooperation. *Neurochem. Int.* **2003**, *43*, 331–338. [CrossRef]
82. Pellerin, L.; Bouzier-Sore, A.K.; Aubert, A.; Serres, S.; Merle, M.; Costalat, R.; Magistretti, P.J. Activity-dependent regulation of energy metabolism by astrocytes: An update. *Glia* **2007**, *55*, 1251–1262. [CrossRef] [PubMed]
83. Dienel, G.A.; Cruz, N.F. Nutrition during brain activation: Does cell-to-cell lactate shuttling contribute significantly to sweet and sour food for thought? *Neurochem. Int.* **2004**, *45*, 321–351. [CrossRef] [PubMed]
84. Brown, A.M.; BaltanTekkök, S.; Ransom, B.R. Energy transfer from astrocytes to axons: The role of CNS glycogen. *Neurochem. Int.* **2004**, *45*, 529–536. [PubMed]
85. Fillenz, M. The role of lactate in brain metabolism. *Neurochem. Int.* **2005**, *47*, 413–417. [CrossRef] [PubMed]
86. Dienel, G.A. Brain lactate metabolism: The discoveries and the controversies. *J. Cereb. Flow Metab.* **2012**, *32*, 1107–1138. [CrossRef] [PubMed]
87. Nehlig, A.; Coles, J.A. Cellular pathways of energy metabolism in the brain: Is glucose used by neurons or astrocytes? *Glia* **2007**, *55*, 1238–1250. [CrossRef] [PubMed]

88. Tsacopoulos, M.; Magistretti, P.J. Metabolic coupling between glia and neurons. *J. Neurosci.* **1996**, *16*, 877–885. [PubMed]
89. Magistretti, P.J.; Pellerin, L. Cellular bases of brain energy metabolism and their relevance to functional brain imaging: Evidence for a prominent role of astrocytes. *Cereb. Cortex* **1996**, *6*, 50–61. [CrossRef] [PubMed]
90. Magistretti, P.J.; Pellerin, L. Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1999**, *354*, 1155–1163. [CrossRef] [PubMed]
91. Nehlig, A.; Wittendorp-Rechenmann, E.; Lam, C.D. Selective uptake of [^{14}C]2-deoxyglucose by neurons and astrocytes: High-resolution microautoradiographic imaging by cellular ^{14}C-trajectography combined with immunohistochemistry. *J. Cereb. Blood Flow Metab.* **2004**, *24*, 1004–1014. [CrossRef]
92. Panov, A.; Orynbayeva, Z.; Vavilin, V.; Lyakhovich, V. Fatty acids in energy metabolism of the central nervous system. *BioMed Res. Int.* **2014**, *2014*. [CrossRef] [PubMed]
93. Lindsay, K.J.; Du, J.; Sloat, S.R.; Contreras, L.; Linton, J.D.; Turner, S.J.; Sadilek, M.; Satrústegui, J.; Hurley, J.B. Pyruvate kinase and aspartate-glutamate carrier distributions reveal key metabolic links between neurons and glia in retina. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 15579–15584. [CrossRef] [PubMed]
94. Zielke, H.R.; Zielke, C.L.; Baab, P.J. Oxidation of ^{14}C-labeled compounds perfused by microdialysis in the brains of free-moving rats. *J. Neurosci. Res.* **2007**, *85*, 3145–3149. [CrossRef] [PubMed]
95. Zielke, H.R.; Zielke, C.L.; Baab, P.J. Direct measurement of oxidative metabolism in the living brain by microdialysis: A review. *J. Neurochem.* **2009**, *109* (Suppl. 1), 24–29. [CrossRef] [PubMed]
96. Caesar, K.; Hashemi, P.; Douhou, A.; Bonvento, G.; Boutelle, M.G.; Walls, A.B.; Lauritzen, M. Glutamate receptor-dependent increments in lactate, glucose and oxygen metabolism evoked in rat cerebellum in vivo. *J. Physiol.* **2008**, *586*, 1337–1349. [CrossRef] [PubMed]
97. Contreras, L.; Satrústegui, J. Calcium signaling in brain mitochondria: Interplay of malate aspartate NADH shuttle and calcium uniporter/mitochondrial dehydrogenase pathways. *J. Biol. Chem.* **2009**, *284*, 7091–7099. [CrossRef] [PubMed]
98. Ivannikov, M.V.; Sugimori, M.; Llinás, R.R. Calcium clearance and its energy requirements in cerebellar neurons. *Cell Calcium* **2010**, *47*, 507–513. [CrossRef] [PubMed]
99. Bak, L.K.; Obel, L.F.; Walls, A.B.; Schousboe, A.; Faek, S.A.; Jajo, F.S.; Waagepetersen, H.S. Novel model of neuronal bioenergetics: Postsynaptic utilization of glucose but not lactate correlates positively with Ca^{2+} signalling in cultured mouse glutamatergic neurons. *ASN Neuro* **2012**, *4*. [CrossRef] [PubMed]
100. Dienel, G.A. Astrocytic energetics during excitatory neurotransmission: What are contributions of glutamate oxidation and glycolysis? *Neurochem. Int.* **2013**, *63*, 244–258. [PubMed]
101. An, J.; Haile, W.B.; Wu, F.; Torre, E.; Yepes, M. Tissue-type plasminogen activator mediates neuroglial coupling in the central nervous system. *Neuroscience* **2014**, *257*, 41–48. [CrossRef] [PubMed]
102. Suzuki, A.; Stern, S.A.; Bozdagi, O.; Huntley, G.W.; Walker, R.H.; Magistretti, P.J.; Alberini, C.M. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* **2011**, *144*, 810–823. [CrossRef] [PubMed]
103. Newman, L.A.; Korol, D.L.; Gold, P.E. Lactate produced by glycogenolysis in astrocytes regulates memory processing. *PLoS ONE* **2011**, *6*, e28427. [CrossRef] [PubMed]
104. Sotelo-Hitschfeld, T.; Niemeyer, M.I.; Mächler, P.; Ruminot, I.; Lerchundi, R.; Wyss, M.T.; Stobart, J.; Fernández-Moncada, I.; Valdebenito, R.; Garrido-Gerter, P.; et al. Channel-mediated lactate release by K-stimulated astrocytes. *J. Neurosci.* **2015**, *35*, 4168–4178. [CrossRef] [PubMed]
105. Ivanov, A.I.; Malkov, A.E.; Waseem, T.; Mukhtarov, M.; Buldakova, S.; Gubkina, O.; Zilberter, M.; Zilberter, Y. Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. *J. Cereb. Blood Flow Metab.* **2014**, *34*, 397–407. [CrossRef] [PubMed]
106. Patel, A.B.; Lai, J.C.; Chowdhury, G.M.; Hyder, F.; Rothman, D.L.; Shulman, R.G.; Behar, K.L. Direct evidence for activity-dependent glucose phosphorylation in neurons with implications for the astrocyte-to-neuron lactate shuttle. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 5385–5390. [CrossRef] [PubMed]
107. Ciofi, P.; Garret, M.; Lapirot, O.; Lafon, P.; Loyens, A.; Prévot, V.; Levine, J.E. Brain-endocrine interactions: A microvascular route in the mediobasal hypothalamus. *Endocrinology* **2009**, *150*, 5509–5519. [CrossRef] [PubMed]
108. Mergenthaler, P.; Lindauer, U.; Dienel, G.A.; Meisel, A. Sugar for the brain: The role of glucose in physiological and pathological brain function. *Trends Neurosci.* **2013**, *36*, 587–597. [CrossRef] [PubMed]

109. Anand, B.K.; Chhina, G.S.; Singh, B. Effect of glucose on the activity of hypothalamic "feeding centers". *Science* **1962**, *138*, 597–598. [CrossRef] [PubMed]
110. Oomura, Y.; Ono, T.; Ooyama, H.; Wayner, M.J. Glucose and osmosensitiveneuronesof the rat hypothalamus. *Nature* **1969**, *222*, 282–284. [CrossRef] [PubMed]
111. Allard, C.; Carneiro, L.; Grall, S.; Cline, B.H.; Fioramonti, X.; Chrétien, C.; Baba-Aissa, F.; Giaume, C.; Pénicaud, L.; Leloup, C. Hypothalamic astroglialconnexins are required for brain glucose sensing-induced insulin secretion. *J. Cereb. Blood Flow Metab.* **2014**, *34*, 339–346. [CrossRef] [PubMed]
112. Lynch, R.M.; Tompkins, L.S.; Brooks, H.L.; Dunn-Meynell, A.A.; Levin, B.E. Localization of glucokinase gene expression in the rat brain. *Diabetes* **2000**, *49*, 693–700. [CrossRef] [PubMed]
113. Danial, N.N.; Gramm, C.F.; Scorrano, L.; Zhang, C.Y.; Krauss, S.; Ranger, A.M.; Datta, S.R.; Greenberg, M.E.; Licklider, L.J.; Lowell, B.B.; et al. BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature* **2003**, *424*, 952–956. [CrossRef] [PubMed]
114. Leloup, C.; Arluison, M.; Lepetit, N.; Cartier, N.; Marfaing-Jallat, P.; Ferré, P.; Pénicaud, L. Glucose transporter 2 (GLUT 2): Expression in specific brain nuclei. *Brain Res.* **1994**, *638*, 221–226. [CrossRef]
115. Arluison, M.; Quignon, M.; Thorens, B.; Leloup, C.; Penicaud, L. Immunocytochemical localization of the glucose transporter 2 (GLUT2) in the adult rat brain. II. Electron microscopic study. *J. Chem. Neuroanat.* **2004**, *28*, 137–146. [CrossRef] [PubMed]
116. Song, Z.; Routh, V.H. Differential effects of glucose and lactate on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes* **2005**, *54*, 15–22. [CrossRef] [PubMed]
117. Venner, A.; Karnani, M.M.; Gonzalez, J.A.; Jensen, L.T.; Fugger, L.; Burdakov, D. Orexin neurons as conditional glucosensors: Paradoxical regulation of sugar sensing by intracellular fuels. *J. Physiol.* **2011**, *589*, 5701–5708. [CrossRef] [PubMed]
118. Schiera, G.; Proia, P.; Alberti, C.; Mineo, M.; Savettieri, G.; di Liegro, I. Neurons produce FGF2 and VEGF and secrete them at least in part by shedding extracellular vesicles. *J. Cell. Mol. Med.* **2007**, *11*, 1384–1394. [CrossRef] [PubMed]
119. Proia, P.; Schiera, G.; Mineo, M.; Ingrassia, A.M.; Santoro, G.; Savettieri, G.; di Liegro, I. Astrocytes shed extracellular vesicles that contain fibroblast growth factor-2 and vascular endothelial growth factor. *Int. J. Mol. Med.* **2008**, *21*, 63–67. [CrossRef] [PubMed]
120. Taylor, A.R.; Robinson, M.B.; Gifondorwa, D.J.; Tytell, M.; Milligan, C.E. Regulation of heat shock protein 70 release in astrocytes: Role of signaling kinases. *Dev. Neurobiol.* **2007**, *67*, 1815–1829. [CrossRef] [PubMed]
121. Wang, S.; Cesca, F.; Loers, G.; Schweizer, M.; Buck, F.; Benfenati, F.; Schachner, M.; Kleene, R. Synapsin I is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes neurite outgrowth and neuronal survival when released via glia-derived exosomes. *J. Neurosci.* **2011**, *31*, 7275–7290. [CrossRef] [PubMed]
122. Lo Cicero, A.; Majkowska, I.; Nagase, H.; di Liegro, I.; Troeberg, L. Microvesicles shed by oligodendroglioma cells and rheumatoid synovial fibroblasts contain aggrecanase activity. *Matrix Biol.* **2012**, *31*, 229–233. [CrossRef] [PubMed]
123. Schiera, G.; di Liegro, C.M.; Saladino, P.; Pitti, R.; Savettieri, G.; Proia, P.; di Liegro, I. Oligodendroglioma cells synthesize the differentiation-specific linker histone H1 and release it into the extracellular environment through shed vesicles. *Int. J. Oncol.* **2013**, *43*, 1771–1776. [PubMed]
124. Schiera, G.; di Liegro, C.M.; di Liegro, I. Extracellular Membrane Vesicles as Vehicles for Brain Cell-to-Cell Interactions in Physiological as well as Pathological Conditions. *Biomed. Res. Int.* **2015**, *2015*. [CrossRef] [PubMed]
125. Lachenal, G.; Pernet-Gallay, K.; Chivet, M.; Hemming, F.J.; Belly, A.; Bodon, G.; Blot, B.; Haase, G.; Goldberg, Y.; Sadoul, R. Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. *Mol. Cell. Neurosci.* **2011**, *46*, 409–418. [CrossRef] [PubMed]
126. Chivet, M.; Hemming, F.; Pernet-Gallay, K.; Fraboulet, S.; Sadoul, R. Emerging role of neuronal exosomes in the central nervous system. *Front. Physiol.* **2012**, *3*. [CrossRef] [PubMed]
127. Fünfschilling, U.; Supplie, L.M.; Mahad, D.; Boretius, S.; Saab, A.S.; Edgar, J.; Brinkmann, B.G.; Kassmann, C.M.; Tzvetanova, I.D.; Möbius, W.; et al. Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* **2012**, *485*, 517–521. [CrossRef] [PubMed]

128. Lee, Y.; Morrison, B.M.; Li, Y.; Lengacher, S.; Farah, M.H.; Hoffman, P.N.; Liu, Y.; Tsingalia, A.; Jin, L.; Zhang, P.W.; et al. Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* **2012**, *487*, 443–448. [CrossRef] [PubMed]
129. Frühbeis, C.; Fröhlich, D.; Kuo, W.P.; Krämer-Albers, E.M. Extracellular vesicles as mediators of neuron-glia communication. *Front. Cell Neurosci.* **2013**, *7*. [CrossRef] [PubMed]
130. Barnes, J.N. Exercise, cognitive function, and aging. *Adv. Physiol. Educ.* **2015**, *39*, 55–62. [CrossRef] [PubMed]
131. Laitman, B.M.; John, G.R. Understanding how exercise promotes cognitive integrity in the aging brain. *PLoS Biol.* **2015**, *13*, e1002300. [CrossRef] [PubMed]
132. Colcombe, S.J.; Erickson, K.I.; Raz, N.; Webb, A.G.; Cohen, N.J.; McAuley, E.; Kramer, A.F. Aerobic fitness reduces brain tissue loss in aging humans. *J. Gerontol. A Biol. Sci. Med. Sci.* **2003**, *58*, 176–180. [CrossRef] [PubMed]
133. Tsai, S.F.; Chen, P.C.; Calkins, M.J.; Wu, S.Y.; Kuo, Y.M. Exercise counteracts aging-related memory impairment: A potential role for the astrocytic metabolic shuttle. *Front. Aging Neurosci.* **2016**, *8*, 57. [CrossRef] [PubMed]
134. Ide, K.; Schmalbruch, I.K.; Quistorff, B.; Horn, A.; Secher, N.H. Lactate, glucose and O_2 uptake in human brain during recovery from maximal exercise. *J. Physiol.* **2000**, *522*, 159–164. [PubMed]
135. Bélanger, M.; Allaman, I.; Magistretti, P.J. Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation. *Cell Metab.* **2011**, *14*, 724–738. [PubMed]
136. Matsui, T.; Ishikawa, T.; Ito, H.; Okamoto, M.; Inoue, K.; Lee, M.C.; Fujikawa, T.; Ichitani, Y.; Kawanaka, K.; Soya, H. Brain glycogen supercompensation following exhaustive exercise. *J. Physiol.* **2012**, *590*, 607–616. [CrossRef] [PubMed]
137. Gibbs, M.E. Role of Glycogenolysis in Memory and Learning: Regulation by Noradrenaline, Serotonin and ATP. *Front. Integr. Neurosci.* **2016**, *9*. [CrossRef] [PubMed]
138. Steinman, M.Q.; Gao, V.; Alberini, C.M. The role of lactate-mediated etabolic coupling between astrocytes and neurons in long-term memory formation. *Front. Integr. Neurosci.* **2016**, *10*. [CrossRef] [PubMed]
139. Cotman, C.W.; Berchtold, N.C.; Christie, L.A. Exercise builds brain health: Key roles of growth factor cascades and inflammation. *Trends Neurosci.* **2007**, *30*, 464–472. [CrossRef] [PubMed]
140. Vivar, C.; Potter, M.C.; van Praag, H. All about running: Synaptic plasticity, growth factors and adult hippocampal neurogenesis. *Curr. Top. Behav. Neurosci.* **2013**, *15*, 189–210. [PubMed]
141. Skriver, K.; Roig, M.; Lundbye-Jensen, J.; Pingel, J.; Helge, J.W.; Kiens, B.; Nielsen, J.B. Acute exercise improves motor memory: Exploring potential biomarkers. *Neurobiol. Learn. Mem.* **2014**, *116*, 46–58. [CrossRef] [PubMed]
142. Mu, J.S.; Li, W.P.; Yao, Z.B.; Zhou, X.F. Deprivation of endogenous brain derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res.* **1999**, *835*, 259–265. [CrossRef]
143. Cirulli, F.; Berry, A.; Chiarotti, F.; Alleva, E. Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. *Hippocampus* **2004**, *14*, 802–807. [CrossRef] [PubMed]
144. Piepmeier, A.T.; Etnier, J.L. Brain-derived neurotrophic factor (BDNF) as a potential mechanism of the effects of acute exercise on cognitive performance. *J. Sport Health Sci.* **2015**, *4*, 14–23. [CrossRef]
145. Gomez-Pinilla, F.; Vaynman, S.; Ying, Z. Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. *Eur. J. Neurosci.* **2008**, *28*, 2278–2287. [CrossRef] [PubMed]
146. Ferris, L.T.; Williams, J.S.; Shen, C.L. The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Med. Sci. Sports Exerc.* **2007**, *9*, 728–734. [CrossRef] [PubMed]
147. Seifert, T.; Brassard, P.; Wissenberg, M.; Rasmussen, P.; Nordby, P.; Stallknecht, B.; Adser, H.; Jacobsen, A.H.; Pilegaard, H.; Nielsen, H.B.; et al. Endurance training enhances BDNF release fron the human brain. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2010**, *298*, R372–R377. [CrossRef] [PubMed]
148. Inoue, K.; Okamoto, M.; Shibato, J.; Lee, M.C.; Matsui, T.; Rakwal, R.; Soya, H. Long-Term Mild, rather than Intense, Exercise Enhances Adult Hippocampal Neurogenesis and Greatly Changes the Transcriptomic Profile of the Hippocampus. *PLoS ONE* **2015**, *10*, e0128720. [CrossRef] [PubMed]
149. Schwartz, A.J.; Brasel, J.; Hintz, R.L.; Mohan, S.; Cooper, D.M. Effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II and IGF-binding protein 3 and its proteolysis in young healthy men. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 3492–3497.

150. Carro, E.; Nuñez, A.; Busiguina, S.; Torres-Aleman, I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J. Neurosci.* **2000**, *20*, 2926–2933. [PubMed]
151. Lista, I.; Sorrentino, G. Biological mechanisms of physical activity in preventing cognitive decline. *Cell. Mol. Neurobiol.* **2010**, *30*, 493–503. [CrossRef] [PubMed]
152. Byth, L.A. Ca^{2+}- and CaMKII-mediated processes in early LTP. *Ann. Neurosci.* **2014**, *21*, 151–153. [CrossRef] [PubMed]
153. Ataei, N.; Sabzghabaee, A.M.; Movahedian, A. Calcium/Calmodulin-dependent Protein Kinase II is a Ubiquitous Molecule in Human Long-term Memory Synaptic Plasticity: A Systematic Review. *Int. J. Prev. Med.* **2015**, *6*. [CrossRef]
154. Slemmon, J.R.; Morgan, J.I.; Fullerton, S.M.; Danho, W.; Hilbush, B.S.; Wengenack, T.M. Camstatins are peptide antagonists of calmodulin based upon a conserved structural motif in PEP-19, neurogranin, and neuromodulin. *J. Biol. Chem.* **1996**, *271*, 15911–15917. [CrossRef] [PubMed]
155. Slemmon, J.R.; Feng, B.; Erhardt, J.A. Small proteins that modulate calmodulin-dependent signal transduction: Effects of PEP-19, neuromodulin, and neurogranin on enzyme activation and cellular homeostasis. *Mol. Neurobiol.* **2000**, *22*, 99–113. [CrossRef]
156. Morgan, M.A.; Morgan, J.I. Pcp4l1 contains an auto-inhibitory element that prevents its IQ motif from binding to calmodulin. *J. Neurochem.* **2012**, *121*, 843–851. [CrossRef] [PubMed]
157. Wang, X.; Xiong, L.W.; El Ayadi, A.; Boehning, D.; Putkey, J.A. The calmodulin regulator protein, PEP-19, sensitizes ATP-induced Ca^{2+} release. *J. Biol. Chem.* **2013**, *288*, 2040–2048. [CrossRef] [PubMed]
158. Di Liegro, C.M.; Schiera, G.; di Liegro, I. Regulation of RNA transport, localization and translation in the nervous system of mammals. *Int. J. Mol. Med.* **2014**, *33*, 747–762. [PubMed]
159. Saladino, P.; di Liegro, C.M.; Proia, P.; Sala, A.; Schiera, G.; Lo Cicero, A.; di Liegro, I. RNA-binding activity of the rat calmodulin-binding PEP-19 protein and of the long PEP-19 isoform. *Int. J. Mol. Med.* **2012**, *29*, 141–145. [PubMed]
160. Dalsgaard, M.K.; Quistorff, B.; Danielsen, E.R.; Selner, C.; Vogelsang, T.; Secher, N.H. A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *J. Physiol.* **2004**, *554*, 571–578. [CrossRef] [PubMed]
161. Van Hall, G.; Strømstad, M.; Rasmussen, P.; Jans, O.; Zaar, M.; Gam, C.; Quistorff, B.; Secher, N.H.; Nielsen, H.B. Blood lactate is an important energy source for the human brain. *J. Cereb. Blood Flow Metab.* **2009**, *29*, 1121–1129. [CrossRef] [PubMed]
162. Coco, M.; Alagona, G.; Rapisarda, G.; Costanzo, E.; Calogero, R.A.; Perciavalle, V. Elevated blood lactate is associated with increased motor cortex excitability. *Somatosens. Mot. Res.* **2010**, *27*, 1–8. [CrossRef] [PubMed]
163. Schiffer, T.; Schulte, S.; Sperlich, B.; Achtzehn, S.; Fricke, H.; Strüder, H.K. Lactate infusion at rest increases BDNF blood concentration in humans. *Neurosci. Lett.* **2011**, *488*, 234–237. [CrossRef] [PubMed]
164. Yang, J.; Ruchti, E.; Petit, J.M.; Jourdain, P.; Grenningloh, G.; Allaman, I.; Magistretti, P.J. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12228–12233. [CrossRef] [PubMed]
165. Bozzo, L.; Puyal, J.; Chatton, J.Y. Lactate modulates the activity of primary cortical neurons through a receptor-mediated pathway. *PLoS ONE* **2013**, *8*, e71721. [CrossRef] [PubMed]
166. Lauritzen, K.H.; Morland, C.; Puchades, M.; Holm-Hansen, S.; Hagelin, E.M.; Lauritzen, F.; Attramadal, H.; Storm-Mathisen, J.; Gjedde, A.; Bergersen, L.H. Lactate receptor sites link neurotransmission, neurovascular coupling, and brain energy metabolism. *Cereb. Cortex.* **2014**, *24*, 2784–2795. [CrossRef] [PubMed]
167. Morland, C.; Lauritzen, K.H.; Puchades, M.; Holm-Hansen, S.; Andersson, K.; Gjedde, A.; Attramadal, H.; Storm-Mathisen, J.; Bergersen, L.H. The lactate receptor, G-protein-coupled receptor 81/hydroxycarboxylic acid receptor 1: Expression and action in brain. *J. Neurosci. Res.* **2015**, *93*, 1045–1055. [CrossRef] [PubMed]
168. Barros, L.F. Metabolic signaling by lactate in the brain. *Trends Neurosci.* **2013**, *36*, 396–404. [CrossRef] [PubMed]
169. Philp, A.; Macdonald, A.L.; Watt, P.W. Lactate-a signal coordinating cell and systemic function. *J. Exp. Biol.* **2005**, *208*, 4561–4575. [CrossRef] [PubMed]
170. Mosienko, V.; Teschemacher, A.G.; Kasparov, S. Is L-lactate a novel signaling molecule in the brain? *J. Cereb. Blood Flow. Metab.* **2015**, *35*, 1069–1075. [CrossRef] [PubMed]
171. Gordon, G.R.; Choi, H.B.; Rungta, R.L.; Ellis-Davies, G.C.; MacVicar, B.A. Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* **2008**, *456*, 745–749. [CrossRef] [PubMed]

172. Lee, D.C.; Sohn, H.A.; Park, Z.Y.; Oh, S.; Kang, Y.K.; Lee, K.M.; Kang, M.; Jang, Y.J.; Yang, S.J.; Hong, Y.K.; et al. A lactate-induced response to hypoxia. *Cell* **2015**, *161*, 595–609. [CrossRef] [PubMed]
173. Rinholm, J.E.; Hamilton, N.B.; Kessaris, N.; Richardson, W.D.; Bergersen, L.H.; Attwell, D. Regulation of oligodendrocyte development and myelination by glucose and lactate. *J. Neurosci.* **2011**, *31*, 538–548. [CrossRef] [PubMed]
174. Arbeláez, A.M.; Cryer, P.E. Lactate and the mechanism of hypoglycemia-associated autonomic failure in diabetes. *Diabetes* **2013**, *62*, 3999–4001. [CrossRef] [PubMed]
175. Maran, A.; Cranston, I.; Lomas, J.; Macdonald, I.; Amiel, S.A. Protection by lactate of cerebral function during hypoglycemia. *Lancet* **1994**, *343*, 16–20. [CrossRef]
176. Veneman, T.; Mitrakou, A.; Mokan, M.; Cryer, P.; Gerich, J. Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans. *Diabetes* **1994**, *43*, 1311–1317. [CrossRef] [PubMed]
177. Boumezbeur, F.; Peterson, K.F.; Cline, G.W.; Mason, G.F.; Behar, K.L.; Shulman, G.I.; Rothman, D.L. The contribution of blood lactate to brain energy metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. *J. Neurosci.* **2010**, *30*, 13983–13991. [CrossRef] [PubMed]
178. Herzog, R.I.; Jiang, L.; Herman, P.; Zhao, C.; Sanganahalli, B.G.; Mason, G.F.; Hyder, F.; Rothman, D.L.; Sherwin, R.S.; Behar, K.L. Lactate preserves neuronal metabolism and function following antecedent recurrent hypoglycemia. *J. Clin. Investig.* **2013**, *123*, 1988–1998. [CrossRef] [PubMed]
179. Brooks, G.A.; Martin, N.A. Cerebral metabolism following traumatic brain injury: New discoveries with implications for treatment. *Front. Neurosci.* **2015**, *8*, 408. [CrossRef]
180. Jalloh, I.; Carpenter, K.L.; Grice, P.; Howe, D.J.; Mason, A.; Gallagher, C.N.; Helmy, A.; Murphy, M.P.; Menon, D.K.; Carpenter, T.A.; et al. Glycolysis and the pentose phosphate pathway after human traumatic brain injury: Microdialysis studies using 1,2-$^{13}C_2$ glucose. *J. Cereb. Blood Flow Metab.* **2015**, *35*, 111–120. [CrossRef] [PubMed]
181. Glenn, T.C.; Martin, N.A.; Horning, M.A.; McArthur, D.L.; Hovda, DA.; Vespa, P.; Brooks, G.A. Lactate: Brain fuel in human traumatic brain injury: A comparison with normal healthy control subjects. *J. Neurotrauma* **2015**, *32*, 820–832. [CrossRef] [PubMed]
182. Gallagher, C.N.; Carpenter, K.L.H.; Grice, P.; Howe, D.J.; Mason, A.; Timofeev, I.; Menon, D.K.; Kirkpatrick, P.J.; Pickard, J.D.; Sutherland, G.R.; et al. The human brain utilizes lactate via the tricarboxylic acid cycle: A 13C-labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain* **2009**, *132*, 2839–2849. [CrossRef] [PubMed]
183. Carpenter, K.L.; Jalloh, I.; Hutchinson, P.J. Glycolysis and the significance of lactate in traumatic brain injury. *Front. Neurosci.* **2015**, *9*. [CrossRef] [PubMed]
184. Bouzat, P.; Sala, N.; Suys, T.; Zerfauth, J.B.; Marques-Vidal, P.; Feihl, F.; Bloch, J.; Messerer, M.; Levivier, M.; Meuli, R.; et al. Cerebral metabolic effects of exogenous lactate supplementation on the injured human brain. *Intensive Care Med.* **2014**, *40*, 412–421. [CrossRef]
185. Lama, S.; Auer, R.N.; Tyson, R.; Gallagher, C.N.; Tomanek, B.; Sutherland, G.R. Lactate storm marks cerebral metabolism following brain trauma. *J. Biol. Chem.* **2014**, *289*, 20200–20208. [CrossRef] [PubMed]
186. Ros, J.; Pecinska, N.; Alessandri, B.; Landolt, H.; Fillenz, M. Lactate reduces glutamate-induced neurotoxicity in rat cortex. *J. Neurosci. Res.* **2001**, *66*, 790–794. [CrossRef] [PubMed]
187. Berthet, C.; Castillo, X.; Magistretti, P.J.; Hirt, L. New evidence of neuroprotection by lactate after transient focal cerebral ischaemia: Extended benefit after intracerebroventricular injection and efficacy of intravenous administration. *Cerebrovasc. Dis.* **2012**, *34*, 329–335. [CrossRef] [PubMed]
188. Castillo, X.; Rosafio, K.; Wyss, M.T.; Drandarov, K.; Buck, A.; Pellerin, L.; Weber, B.; Hirt, L. A probable dual mode of action for both L- and D-lactate neuroprotection in cerebral ischemia. *J. Cereb. Blood Flow Metab.* **2015**, *35*, 1561–1569. [CrossRef] [PubMed]
189. Detka, J.; Kurek, A.; Kucharczyk, M.; Głombik, K.; Basta-Kaim, A.; Kubera, M.; Lasoń, W.; Budziszewska, B. Brain glucose metabolism in an animal model of depression. *Neuroscience* **2015**, *295*, 198–208. [CrossRef] [PubMed]
190. Bosoi, C.R.; Zwingmann, C.; Marin, H.; Parent-Robitaille, C.; Huynh, J.; Tremblay, M.; Rose, C.F. Increased brain lactate is central to the development of brain edema in rats with chronic liver disease. *J. Hepatol.* **2014**, *60*, 554–560. [CrossRef] [PubMed]

191. Bosoi, C.R.; Rose, C.F. Elevated cerebral lactate: Implications in the pathogenesis of hepatic encephalopathy. *Metab. Brain Dis.* **2014**, *29*, 919–925. [CrossRef] [PubMed]
192. Redjems-Bennani, N.; Jeandel, C.; Lefebvre, E.; Blain, H.; Vidailhet, M.; Guéant, J.L. Abnormal substrate levels that depend upon mitochondrial function in cerebrospinal fluid from Alzheimer patients. *Gerontology* **1998**, *44*, 300–304. [CrossRef] [PubMed]
193. Xiang, Y.; Xu, G.; Weigel-Van Aken, K.A.K. Lactic Acid Induces Aberrant Amyloid Precursor Protein Processing by Promoting Its Interaction with Endoplasmic Reticulum Chaperone Proteins. *PLoS ONE* **2010**, *5*, e13820. [CrossRef] [PubMed]
194. Monteiro-Junior, R.S.; Cevada, T.; Oliveira, B.R.; Lattari, E.; Portugal, E.M.; Carvalho, A.; Deslandes, A.C. We need to move more: Neurobiological hypotheses of physical exercise as a treatment for Parkinson's disease. *Med. Hypotheses* **2015**, *85*, 537–541. [CrossRef] [PubMed]
195. Herbst, E.A.F.; Holloway, G.P. Exercise training normalizes mitochondrial respiratory capacity within the striatum of R6/1 model of Huntington's Disease. *Neuroscience* **2015**, *303*, 515–523. [CrossRef] [PubMed]
196. Wens, I.; Dalgas, U.; Vandennabeele, F.; Verboven, K.; Hansen, D.; Deckx, N.; Cools, N.; Eijnde, B.O. High intensity aerobic and resistance exercise can improve glucose tolerance in persons with Multiple Sclerosis: A randomized controlled trial. *Am. J. Phys. Med. Rehabil.* **2016**. in press. [CrossRef] [PubMed]
197. Bharadwaj, S.; Venkatraghavan, L.; Mariappan, R.; Ebinu, J.; Meng, Y.; Khan, O.; Tung, T.; Reyhani, S.; Bernstein, M.; Zadeh, G. Serum lactate as a potential biomarker of non-glial brain tumors. *J. Clin. Neurosci.* **2015**, *22*, 1625–1627. [CrossRef] [PubMed]