Synaptosomal Lactate Dehydrogenase Isoenzyme Composition Is Shifted toward Aerobic Forms in Primate Brain Evolution

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Key Words

Lactate dehydrogenase · Strepsirrhines · Haplorhines · Brain · Metabolism · Synaptosome

Abstract

With the evolution of a relatively large brain size in haplorhine primates (i.e. tarsiers, monkeys, apes, and humans), there have been associated changes in the molecular machinery that delivers energy to the neocortex. Here we investigated variation in lactate dehydrogenase (LDH) expression and isoenzyme composition of the neocortex and striatum in primates using quantitative Western blotting and isoenzyme analysis of total homogenates and synaptosomal fractions. Analysis of isoform expression revealed that LDH in synaptosomal fractions from both forebrain regions shifted towards a predominance of the heart-type, aerobic isoform LDH-B among haplorhines as compared to strepsirrhines (i.e. lorises and lemurs), while in the total homogenate of the neocortex and striatum there was no significant difference in LDH isoenzyme composition between the primate suborders. The largest increase occurred in synapse-associated LDH-B expression in the neocortex, with

an especially remarkable elevation in the ratio of LDH-B/LDH-A in humans. The phylogenetic variation in the ratio of LDH-B/LDH-A was correlated with species-typical brain mass but not the encephalization quotient. A significant LDH-B increase in the subneuronal fraction from haplorhine neocortex and striatum suggests a relatively higher rate of aerobic glycolysis that is linked to synaptosomal mitochondrial metabolism. Our results indicate that there is a differential composition of LDH isoenzymes and metabolism in synaptic terminals that evolved in primates to meet increased energy requirements in association with brain enlargement.

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Abbreviations used in this paper

GFAP	glial fibrillary acidic protein
HIF-1	hypoxia-inducible factor 1
LDH	lactate dehydrogenase
MCT	monocarboxylic acid transporter
NBT	nitroblue tetrazolium
NWM	New World monkeys
OWM	Old World monkeys
PMS	phenazine methosulphate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SYP	synaptophysin

Morris Goodman is deceased (November 15, 2010).

Introduction

During the course of primate evolution, changes in metabolic genes have accompanied brain size expansion [Horrobin, 1998; Grossman et al., 2001, 2004; Uddin et al., 2008; Goodman and Sterner, 2010]. There are several strategies for meeting the energetic demands of a large brain and they can be manifested differentially across taxa [Barrickman and Lin, 2010; Navarrete et al., 2011]. One metabolic strategy involves greater use of lactate as a neuronal fuel. It has been demonstrated that the conversion of lactate to and from pyruvate is governed by specific lactate dehydrogenase (LDH) isoforms, thereby forming a highly adaptable metabolic intermediate system [Brooks, 2002; Castro et al., 2009]. Furthermore, it has been suggested that the brain's ability to produce and use lactate can be locally regulated by changing LDH-A/LDH-B gene activity ratios and controlling the subunit composition of LDH isoenzymes, allowing the brain to optimize the use of energy resources and metabolism [Ross et al., 2010].

Because LDH catalyzes the interconversion of pyruvate to lactate and regulates the levels of these metabolites in accordance with oxygen availability, it functions at the junction between glycolysis, the metabolic pathway that converts glucose into pyruvate, and the tricarboxylic acid cycle, a series of enzyme-catalyzed chemical reactions that form a key part of aerobic respiration in cells. The protein subunits of LDH (L-lactate:NAD⁺ oxidoreductase, EC 1.1.1.27) exist as 2 major structural forms which are each encoded by separate genes, usually referred to as LDH-A and LDH-B, which give rise to 5 different isoenzymes of the tetrameric molecule in vertebrates: B4, B3A, A2B2, BA3, and A4. The differences in the properties of the LDH isoenzymes are dependent on their subunit composition and are most exaggerated between the homotetramers A4 (LDH-5) and B4 (LDH-1).

According to the compositional ratio of the 2 subunits in a tetramer and its 5 isoenzymes *in vivo*, the total LDH activity exhibits distinct physical and catalytic properties. The high concentrations of pyruvate present in anaerobic tissues can be effectively reduced to lactate by the A4 and A3B1 isoenzymes, while the B4 and B3A1 isoenzymes are inhibited by such high levels of pyruvate. On the other hand, the B4 and B3A1 isoenzymes are able to catalyze efficiently the interconversion of low concentrations of pyruvate and lactate, as present in aerobic tissues, because of their high affinity for these substrates. Pyruvate reconverted from lactate is then further converted to acetyl-

CoA and irreversibly committed to entering the citric acid cycle.

The observation of variable LDH isoenzyme distributions in different tissues led to hypotheses regarding possible functions [Dawson et al., 1964; Fondy and Kaplan, 1965]. The two most common isoforms, LDH-A and LDH-B, have overlapping tissue expression across mammals, with LDH-B predominantly expressed in the heart and LDH-A expressed in skeletal muscle [Nisselbaum and Bodansky, 1959; Plagemann et al., 1960; Vesell, 1961; Vesell and Bearn, 1961; Charpentier and Goutefongea, 1964; Hinks and Masters, 1964, 1965; Latner and Skillen, 1964; Goodman et al., 1969; Koen and Goodman, 1969; Beebe and Carty, 1982; Milne and Doxey, 1987].

The expression profile of these two isoenzymes in the brain also shows marked variation across species and more pronounced differences in certain regions of the brain than in others [Lowenthal et al., 1961; Nisselbaum and Bodansky, 1961; Syner and Goodman, 1966; Goodman et al., 1969; Koen and Goodman, 1969]. Among haplorhine primates with relatively large brain sizes, the shift from LDH-A to LDH-B is especially pronounced in the neocortex and corpus callosum as compared to smaller-brained strepsirrhines [Goodman et al., 1969]. For instance, it has been shown that LDH-A is richest in the strepsirrhine *Lorisoida*, especially in their cerebral cortical regions, which have 3–4 times more LDH-A than the aerobic B type of LDH, whereas LDH-B is more highly expressed in the cerebral cortex of haplorhine species, including humans, other apes, and monkeys [Lowenthal et al., 1961; Nisselbaum and Bodansky, 1961; Syner and Goodman, 1966; Goodman et al., 1969; Koen and Goodman, 1969].

Furthermore, recent studies on the distribution of LDH-A and LDH-B support the theory of metabolic compartmentalization and have reported that there is selective, or even exclusive, enrichment of LDH-B in neurons and LDH-A in astrocytes [Bittar et al., 1996; Laughon et al., 2000; Pellerin, 2003]. For the most part, however, these studies have determined the expression of RNA messages for the A and B subunits rather than the actual level and distribution of the isoenzymes in brain cells.

The current research explores variation in LDH isoenzyme expression among species to determine the pattern of metabolic changes in the evolution of the forebrain of primates. Specifically, we examined LDH isoenzyme expression in the synaptosomal, neuronal enriched fraction, as well as in total homogenates from the neocortex and striatum of different primate species.

Materials and Methods

Sources of Samples

Frozen brain samples from nonhuman primates were acquired from naturally deceased or humanely euthanized animals from various zoos and research facilities. All protocols were approved by the relevant Institutional Animal Care and Use Committees or Scientific Advisory Committees. All brains were collected within 14 h of the animal's death. No pathology was observed in the brain on routine examination and no individual showed neurological symptoms before death. Each brain was stored at -80°C prior to use in this study. The taxonomic composition and full details of the age and sex distribution of the samples are shown in table 1. For each individual, 2 independent tissue samples (approximately 100–150 mg each) were dissected from the right parietal cortex and striatum without thawing. Both samples from each region for each individual were processed independently and then used for protein extraction and further analysis. At least 2 independent analyses were performed on isolated synaptosomes and 2 pieces of the parietal cortex were taken for each experimental setup and for each biochemical analysis.

For statistical analyses, the sample was divided into 3 phylogenetic groups: strepsirrhines (including lemurs and lorises), 'monkeys' [including New World monkeys (NWM) and Old World monkeys (OWM)], and hominids (including humans and chimpanzees). Even though NWM and OWM are paraphyletic, we pooled data from these species for certain statistical analyses in order to increase the sample size and because they share a somewhat similar brain size. We also analyzed the results divided into the primate suborders Strepsirrhines and Haplorhines.

Materials

L-Lactate (lithium salt) and all other chemicals were purchased from Sigma Chemical (St. Louis, Mo., USA). The NativePAGE Novex Bis-Tris Gel System kit was purchased from Invitrogen (Carlsbad, Calif., USA). All reagents and chemicals were of the highest analytical grade quality. Solutions for the protein assay were purchased from Bio-Rad (Hercules, Calif., USA).

Preparation of Synaptosomal-Enriched Fractions

Synaptosomal fractions were prepared from the dissected brain samples by 2 different methods: (1) sucrose density gradient centrifugation, and (2) synaptic vesicle immunoisolation (data not shown in this paper). Detailed procedures for the preparation of synaptosome-enriched fractions can be found in the online supplementary material (www.karger.com/doi/10.1159/000358581 for all online suppl. material). These two experimental protocols were compared mainly on the basis of protein yield after the purification procedure. Synaptosomal purity was assessed by Western blot analysis of the presynaptic marker synaptophysin (SYP) and the glial marker glial fibrillary acidic protein (GFAP). To quantify the extent of LDH isoenzyme colocalization, we scanned the films and measured the band densities using Scion Image software (Scion Corp., Frederick, Md., USA).

Isoenzyme Analysis: Electrophoresis and Staining

LDH isoenzyme patterns were determined by previously published methods [Leiblich et al., 2006]. Briefly, the LDH isoenzymes in the subcellular fractions from the cerebral cortex samples were separated on the NativePAGE Novex Bis-Tris Gel System (Invitrogen).

Regions of LDH activity were made visible using a staining solution containing 0.1 M sodium lactate, 1.5 mM NAD, 0.1 M Tris-HCl (pH 8.6), 10 mM NaCl, 5 mM MgCl_2 , 0.03 mg/ml phenazine methosulphate (PMS), and 0.25 mg/ml nitroblue tetrazolium (NBT). The assay relies on the conversion of lactate to pyruvate, with the production of NADH and H^+ . The NADH then reduces the PMS, which in turn reduces the NBT to yield an insoluble product, diformazan. Proteins extracted from human heart and skeletal muscles served as positive controls and were obtained from Novus Biologicals. LDH isoenzyme bands were identified by comparison of retention times to standards. Individual bands were quantified by densitometry. Values for individual isoenzymes are expressed as percents of the total isoenzyme (the sum of the densitometry of all isoenzymes in that sample) and reflect the mean $\pm$ SD of 3 gels.

Western Blot Analysis

Samples obtained from all protocols were analyzed using Western blots with 4–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (NP0322BOX; Invitrogen). Western blot assays were performed as described previously [Sherwood et al., 2010]. The following primary antibodies were used for immunoblotting: anti-LDH-A (1/1,000, sc-27230; Santa Cruz Biotechnology), anti-LDH-B (1/500, NB100-79987; Novus Biologicals), and anti-LDH (1/1,000, sc-133123; Santa Cruz Biotechnology). Antibodies were characterized in detail before being used for research (online suppl. material). The blots were also probed with antibodies against cell-specific marker proteins: anti-GFAP (glial marker, 1/1,000, sc-9065; Santa Cruz Biotechnology) and anti-SYP (presynaptic neuronal marker, 1/1,000, ab14692; Abcam). For the detection of antibodies, appropriate peroxidase-conjugated secondary antibodies were used in conjunction with enhanced chemiluminescence (Amersham Pharmacia Biosciences) to obtain images saved on film. The signals were quantitatively evaluated using Scion Image software. Equal protein loading was confirmed with anti- β -actin antibody (1/1,000, sc-1616; Santa Cruz Biotechnology). Protein bands were scanned using an EPSON Perfection 4870 Photo scanner and quantitatively analyzed using Scion Image software.

Representative Western Blots and Zymograms

The samples for the representative Western blots as well as the zymograms presented in figures 1–4 were comprised of a mixture of total homogenates pooled from the species-represented larger phylogenetic group. To account for blot-to-blot variation in exposure and film development, 3 concentrations of a blotting standard were loaded onto each gel. The standard comprised a mixture of protein samples from 2 human inferior frontal gyri. The intensity of the bands for each unknown sample was normalized to this standard and quantified using Scion Image software.

Enzyme-Linked Immunosorbent Assay

For quantitative determination of the LDH-A and LDH-B relative concentrations in total brain homogenates and subcellular fractions, the quantitative enzyme immunoassay technique was used with commercially available antibodies against 2 LDH isoforms. Details of the protocol can be found in the online supplementary material.

Table 1. Characteristics of the sample, brain regions examined, and methods used

Species	Age, years	Sex	Brain regions	Experimental methods		
				Western blot	isoenzyme analysis	ELISA
Lorises						
Red slender loris (<i>Loris tardigradus</i>)	18.4	M	neocortex striatum	yes no	yes no	no no
Pygmy slow loris (<i>Nycticebus pygmaeus</i>)	14	M	neocortex striatum	yes no	yes no	no no
Pygmy slow loris (<i>Nycticebus pygmaeus</i>)	10	M	neocortex striatum	yes yes	yes yes	yes yes
Lemurs						
Blue-eyed black lemur (<i>Eulemur flavifrons</i>)	18.1	F	neocortex striatum	yes no	yes no	no no
Ring-tailed lemur (<i>Lemur catta</i>)	15	F	neocortex striatum	yes no	yes no	no no
Ring-tailed lemur (<i>Lemur catta</i>)	24	F	neocortex	yes	yes	yes
New World Monkeys						
White-fronted spider monkey (<i>Ateles belzebuth</i>)	36	F	neocortex striatum	yes no	yes no	no no
White-faced saki (<i>Pithecia pithecia</i>)	10	F	neocortex striatum	yes yes	yes yes	yes yes
Squirrel monkey (<i>Saimiri sciureus</i>)	30	F	neocortex striatum	yes yes	yes yes	yes yes
Old World Monkeys						
Olive baboon (<i>Papio anubis</i>)	6.5	F	neocortex striatum	yes no	yes no	no no
Rhesus macaque (<i>Macaca mulatta</i>)	23.3	F	neocortex striatum	yes yes	yes yes	yes yes
Rhesus macaque (<i>Macaca mulatta</i>)	24.5	M	neocortex striatum	yes yes	yes yes	yes yes
Rhesus macaque (<i>Macaca mulatta</i>)	13.2	M	neocortex striatum	yes yes	yes yes	yes yes
Rhesus macaque (<i>Macaca mulatta</i>)	15.67	F	neocortex striatum	yes yes	yes yes	yes yes
Pig-tailed macaque (<i>Macaca nemestrina</i>)	15.6	M	neocortex striatum	yes no	yes no	no no
Hominids						
Chimpanzee (<i>Pan troglodytes</i>)	31.2	M	neocortex striatum	yes no	yes no	no no
Chimpanzee (<i>Pan troglodytes</i>)	22.4	F	neocortex striatum	yes yes	yes yes	yes yes
Chimpanzee (<i>Pan troglodytes</i>)	26.4	M	neocortex striatum	yes yes	yes yes	yes yes
Chimpanzee (<i>Pan troglodytes</i>)	22.9	M	neocortex striatum	yes yes	yes yes	yes yes
Chimpanzee (<i>Pan troglodytes</i>)	31.2	M	neocortex striatum	yes no	yes no	yes no
Human (<i>Homo sapiens</i>)	73	M	neocortex striatum	yes no	yes no	no no
Human (<i>Homo sapiens</i>)	78	F	neocortex striatum	yes no	yes no	no no
Human (<i>Homo sapiens</i>)	46.4	F	neocortex striatum	yes yes	yes yes	yes yes
Human (<i>Homo sapiens</i>)	51.2	M	neocortex striatum	yes yes	yes yes	yes yes
Human (<i>Homo sapiens</i>)	49.4	M	neocortex striatum	yes yes	yes yes	yes yes

Calculation of Aerobic Status, the Percent Value of LDH Subunits, and Statistical Analysis

The LDH-B/LDH-A ratio, or aerobic status [Koen and Goodman, 1969, 1971], was calculated as the densitometry of LDH-B expression divided by LDH-A, adjusted by loading control levels. The percent value of LDH subunits in the zymograms was calculated from the measured intensities of the LDH bands. A nonparametric Mann-Whitney U test was used to determine significant differences between the strepsirrhine and haplorhine groups. A Kruskal-Wallis one-way analysis of variance test was used to examine differences between LDH expressions in the following primate groups: strepsirrhines, 'monkeys', and hominids. PAST statistical software [Hammer et al., 2001] was used for analyses. All data are expressed as means $\pm$ SD. Pearson correlations were conducted on the optical density values from isoenzyme, Western blot, and enzyme-linked immunosorbent assay (ELISA) analyses to investigate their relationship with protein expression in fractions of the neocortex and striatum. $\alpha \leq 0.05$ was considered statistically significant.

Results

Expression Changes in LDH-A and LDH-B in Synaptosomal Fractions

We used Western blotting and ELISA to identify phylogenetic differences in LDH isoform expression profiles in synaptosomal fractions (fig. 1). The two methods concordantly revealed that LDH-B levels in synaptosomal fractions from the neocortex and striatum varied significantly across species. In contrast, LDH-A levels also varied across species but showed an opposite pattern of decreasing expression in those species where LDH-B levels were increased. Quantitative Western blot analysis demonstrated that the neocortical synaptosomal LDH-B expression level was elevated 2-fold in NWM, 6-fold in OWM, and 12-fold in chimpanzees and humans as compared to that in strepsirrhines. By contrast, the LDH-A level was decreased 1.5-fold in NWM, 2-fold in OWM, and 4-fold in chimpanzees and humans relative to that in strepsirrhines (fig. 1a, c).

In the striatum there were also significant differences in LDH-B and LDH-A synaptosomal protein levels among groups, but the magnitude of this variation was not as remarkable as that observed in the neocortex. The LDH-B expression level was elevated approximately 2-fold in OWM, chimpanzees, and humans, while the LDH-A level was decreased 1.3-fold in NWM, 1.6-fold in OWM, and 2.8-fold in chimpanzees and humans as compared to strepsirrhines (fig. 1b, d). These results were further corroborated by ELISA (fig. 1e).

The differences among species were not as pronounced when total tissue homogenates were used in

the analyses. For example, Western blot analysis of total homogenates from the neocortex detected a relatively small difference in LDH-B expression level between the human and chimpanzee groups versus strepsirrhines (1.7-fold increase $p = 0.11$, Kruskal-Wallis test) (fig. 2a–d). A higher LDH-B expression level was identified by ELISA in total homogenates of haplorhine neocortex (by 96%) and striatum (by 81%) as compared to strepsirrhine primates ($p \leq 0.05$, Mann-Whitney U tests) (fig. 2e, f). Additionally, no significant differences in LDH-A between the strepsirrhine and haplorhine groups were found in either neocortex or striatum from total homogenates ($p = 0.07$ and $p = 0.25$, Mann-Whitney U test accordingly for the neocortex and striatum) (fig. 2a–d).

LDH Isoenzyme Patterns in the Synaptosomal Fraction Shifted toward Aerobic Forms of the Enzyme in the Neocortex and Striatum of Haplorhine Primates

Isoenzyme analysis of synaptosomal fractions and total homogenates revealed 5 isoenzyme bands corresponding to the 5 possible tetrameric LDH isoforms (fig. 3, 4). Densitometric analysis showed that both aerobic and anaerobic forms were present in synaptosomal fractions from strepsirrhines and NWM and OWM, whereas the anaerobic forms, LDH-BA3 and LDH-A4, were not found, or were found in trace concentrations, in the neocortex (fig. 3a, b) and striatum synaptosomes of humans and chimpanzees (fig. 4a, b).

Regional differences were apparent in the levels of synaptosomal LDH isoforms among primate taxa. As expected, synaptosomal fractions from strepsirrhine neocortex (fig. 3a, b) predominantly showed expression of the LDH-BA3 (44.3 ± 4.3) isoenzyme, with a weaker expression of LDH-B4 (14.2 ± 2.6) and LDH-B3A1 (6.7 ± 0.9). In contrast, synaptosomal fractions from chimpanzees and humans displayed LDH-B4 (46.3 ± 11.9) and LDH-B3A1 (27.5 ± 6.8) as major forms, with no detectable levels of LDH-A3B1 or LDH-A4 (fig. 3c, d). Synaptosomal fractions from both NWM and OWM displayed an equal balance between the percentages of aerobic and anaerobic isoforms (fig. 3e, f).

In synaptosomes derived from the striatum of strepsirrhines, the highest level of expression was for the LDH anaerobic form LDH-B1A3 (29.4 ± 6.3) (fig. 4a, b), and the lowest expression was for the LDH aerobic form LDH-B3A1 (11.0 ± 2.7) (fig. 4c, d). The LDH isoenzyme composition of chimpanzee and human striatal synaptosome samples showed an increase in the amount of aerobic B forms LDH-B4 (31.9 ± 5.9) and LDH-B3 A1 ($29.9 \pm$

Fig. 1. Comparative analyses of LDH isoenzyme expression levels in synaptosomal fractions from the neocortex and striatum. Representative immunoblots show LDH isoenzymes in the neocortical (a) and striatal (b) synaptosomal fractions. An isoform-indifferent antibody was utilized to detect the total level of LDH and as an internal loading control. c, d The densitometry-evaluated levels of LDH-A and LDH-B proteins, as determined by Western blot, are

shown. Line graphs show the relative LDH isoenzyme levels as measured by ELISA in neocortical (e) and striatal (f) synaptosomes. * and # indicate $p \leq 0.05$ for Mann-Whitney U tests between strepsirrhines and haplorhines for fractional LDH-A and LDH-B levels, respectively. ^ and & indicate $p \leq 0.05$ for Kruskal-Wallis tests among strepsirrhines, 'monkeys', and chimpanzees and humans for LDH-A and LDH-B, respectively.

Fig. 2. Comparative analyses of LDH isoenzyme expression levels in total homogenates from the neocortex and striatum. Representative immunoblots showing LDH isoenzymes in the neocortical (**a**) and striatal (**b**) total homogenates. An isoform-indifferent antibody was utilized to detect the total level of LDH and to serve as an internal loading control. **c, d** The densitometry-evaluated level of LDH-A and LDH-B proteins, as determined by Western blot,

are shown. Line graphs show the relative LDH isoenzyme levels as measured by ELISA in neocortical (**e**) and striatal (**f**) total homogenates. # indicates $p \leq 0.05$ for Mann-Whitney U test between strepsirrhines and haplorhines for fractional LDH-B levels; & indicates $p \leq 0.05$ for Kruskal-Wallis test among strepsirrhines, 'monkeys', and chimpanzees and humans for LDH-B.

Fig. 3. LDH isoenzyme patterns from the synaptosomal neocortical fractions and total homogenates. Representative zymograms from two fractions, synaptosomal (**a**) and total homogenates (**b**), isolated from the neocortex. The LDH isoenzyme tetrameric composition (**b**) indicates the number of A (anaerobic) and B (aerobic) subunits that constitute each isoenzyme. Quantification of the relative amount of protein subunits was achieved using scanning gels as described in the Materials and Methods section. The values ob-

tained by densitometric analysis of the blotted LDH isoenzymes are reported as isoform compositions (**c**, **d**) and as proportions of predominantly A or B forms, or A2B2 forms (**e**, **f**). * indicates that the difference in the values of strepsirrhines versus haplorhines was significant (Mann-Whitney U test, $p \leq 0.05$). # indicates that the Kruskal-Wallis test showed significant differences among the following primate groups: strepsirrhines, 'monkeys', and chimpanzees and humans ($p \leq 0.05$).

4.2), with a minor amount of anaerobic forms LDH-A3B1 (6.7 ± 0.8) and LDH-A4 (4.9 ± 0.5) (fig. 4c-f).

In total homogenates, the occurrence of the 5 AB tetramers in all primates was evident, with a predominance of LDH-A4 in strepsirrhine neocortex (28.3 ± 5.2) (fig. 3) and striatum (33.4 ± 5.5) (fig. 4). Both NWM and OWM have maintained almost the same level of LDH-A4 in the neocortex (22.2 ± 5.6) and striatum (29.3 ± 4.6); chimpanzees and humans, on the other hand, predominantly demonstrated expression of LDH-B4 in the neocortex (30.6 ± 5.7) and striatum (22.7 ± 6.2) (fig. 3, 4).

Phylogenetic differences were observed in total neocortical homogenates from chimpanzees and humans for LDH-B4, LDH-B3A (2.1- and 1.6-fold) and LDH-A4 and LDH-A3B (2- and 1.9-fold) as compared to strepsirrhines ($p \leq 0.05$, Mann-Whitney U test, and $p \leq 0.05$, Kruskal-Wallis test, accordingly for aerobic and anaerobic LDH isoforms) (fig. 3). In the striatum, the level of LDH-B4 was increased (1.9-fold) in haplorhines as compared to strepsirrhine primates, but this difference did not reach statistical significance ($p = 0.15$, Mann-Whitney U test) (fig. 4).

Ratios of LDH-B/LDH-A in Synaptosomal Fractions from the Neocortex

The polypeptide ratio LDH-B/LDH-A signifies aerobic status [Koen, 1969, 1971], and phylogenetic differences in the proportions of two kinds of LDH in primate brain regions have been shown previously [Goodman et al., 1969]. Here we calculated the polypeptide ratio of single isoforms, LDH-B/LDH-A (Western blot, isoenzyme analysis, and ELISA), for neocortical and striatal brain fractions (table 2). As estimated by these three different methods, LDH-B/LDH-A ratios differed markedly in the synaptosomal neocortical fractions among primate taxa (table 2).

The average ratio, as measured by Western blot, increased 5- to 10-fold across taxa, from 0.195 in strepsirrhines to 0.484 in NWM, 3.256 in OWM, and 9.194 in chimpanzees and humans. The LDH-B/LDH-A ratio in the synaptosome obtained from the striatum as measured by Western blot also varied 2- to 3- fold with phylogeny, from a ratio of 0.520 in strepsirrhines to 0.663 in NWM, 1.588 in OWM, and 3.270 in chimpanzees and humans.

When results from the Western blotting and ELISA methods were compared, a high correlation of the LDH-B/LDH-A ratio in synaptosomal fractions was obtained from the neocortex ($r = 0.998$, $p = 0.0001$) and striatum ($r = 0.985$, $p = 0.0003$). It should be noted that we were unable to calculate the ratio of LDH-B/LDH-A by using

Table 2. Quantification of the relative ratio of LDH-B/LDH-A in primate neocortex and striatum (Western blot, isoenzyme analysis, and ELISA of the total homogenates and synaptosomal fractions)

Species group	Total homogenates				Synaptosomal fractions							
	Western blot		isoenzyme analysis		ELISA		Western blot		isoenzyme analysis		ELISA	
	neocortex	striatum	neocortex	striatum	neocortex	striatum	neocortex	striatum	neocortex	striatum	neocortex	striatum
Lorises	0.633±0.089	0.753±0.073	0.631±0.062	0.589±0.049	0.461±0.052	0.571±0.049	0.226±0.034	0.641±0.076	0.408±0.059	0.652±0.034	0.153±0.019	0.428±0.075
Lemurs	0.608±0.175	0.588±0.066	0.554±0.057	0.435±0.034	0.572±0.028	0.568±0.054	0.164±0.030	0.398±0.035	0.328±0.031	0.652±0.048	0.089±0.016	0.283±0.032
Nonhuman primates	0.619±0.163	0.603±0.094	0.562±0.075	0.635±0.061	0.587±0.035	0.593±0.080	0.484±0.035	0.663±0.087	0.827±0.080	1.260±0.278	0.516±0.058	0.512±0.061
OWM	1.136±0.346	0.898±0.097	0.944±0.131	0.690±0.078	1.496±0.364	1.155±0.316	3.256±0.549	1.588±0.101	1.324±0.337	1.753±0.105	3.184±0.251	1.572±0.175
Chimpanzees	1.538±0.308	1.283±0.420	1.837±0.221	0.787±0.138	1.472±0.496	1.292±0.479	6.640±0.768	2.541±0.271	Undefined	3.209±0.301	5.853±0.696	2.191±0.426
Humans	1.341±0.351	1.078±0.363	2.735±0.438	1.314±0.335	1.355±0.387	1.161±0.441	11.747±2.570	3.998±0.319	Undefined	4.264±0.539	9.914±1.031	3.512±0.436

Fig. 4. LDH isoenzyme patterns from the synaptosomal striatal fractions and total homogenates. Representative zymograms from two fractions, synaptosomal (**a**) and total homogenates (**b**), isolated from the primate striatum. The LDH isoenzyme tetrameric composition (**b**) indicates the number of A (anaerobic) and B (aerobic) subunits that constitute each isoenzyme. Quantification of the relative amount of protein subunits was achieved using scanning gels as described in the Materials and Methods section. The

values obtained by densitometric analysis of the blotted LDH isoenzymes are reported as isoform compositions (**c**, **d**) and as proportions of predominantly A or B forms, or A2B2 forms (**e**, **f**). * indicates that the difference in the values of strepsirrhines versus haplorhines was significant (Mann-Whitney U test, $p \leq 0.05$). # indicates that the Kruskal-Wallis test showed significant differences among the following primate groups: strepsirrhines, 'monkeys', and chimpanzees and humans ($p \leq 0.05$).

Fig. 5. Analysis of the LDH ratio versus brain mass. Graphs show the association between the ratio of LDH-B/LDH-A expression in total homogenates (**a**) and synaptosomal (**b**) fractions and brain mass across primates. A cubic spline was fitted to the data from total homogenates and the best-fit quadratic function was fitted to the data from synaptosomes.

isoenzyme analysis due to an inability to determine anaerobic A forms of LDH in the synaptosomal fractions from the neocortex of chimpanzees and humans.

Ratio of LDH-B/LDH-A Expression in Total Homogenates and Synaptosomal Fractions versus Brain Size

We examined the association between the ratio of LDH-B/LDH-A expression in total homogenates and synaptosomal fractions versus brain size in our comparative sample of primates. For this analysis, we calculated species' mean LDH-B/LDH-A ratios based on Western blot and we obtained measures of the species' average brain mass and encephalization quotient (EQ) from Boddy et al. [2012]. For the 3 individuals in our sample that did not have a brain mass or EQ reported in the Boddy et al. [2012] dataset, we used brain masses from other conspecifics that we have in our brain collection and we calculated an average EQ from the congeners in the Boddy et al. [2012] dataset. To model the relationship, we fit separate linear, quadratic, and cubic polynomial regressions to the LDH-B/LDH-A ratio values as a function of brain mass or EQ. Results showed that all three polynomial regressions for LDH-B/LDH-A ratio data against brain mass were significant and quadratic functions had the lowest *p* values and therefore were selected as the best fit for the data (fig. 5). The quadratic models for the synaptosomal LDH-B/LDH-A ratio were more highly correlated with brain mass (neocortex: $r^2 = 0.925$, $F_{2,9} = 55.8$,

$p < 0.001$; striatum: $r^2 = 0.970$, $F_{2,4} = 65.7$, $p = 0.001$) than the total homogenate values (neocortex: $r^2 = 0.804$, $F_{2,9} = 18.5$, $p = 0.001$; striatum: $r^2 = 0.904$, $F_{2,4} = 18.8$, $p = 0.009$). None of the polynomial regressions of any LDH-B/LDH-A ratio data against EQ were significant.

Discussion

Brain tissue has an exceptionally high metabolic rate and, unlike in other tissues and organs, cerebral metabolism may not be reduced without causing irreversible brain damage. Thus, it is often asserted that an evolutionary increase in brain size requires compensatory metabolic adaptations [Armstrong, 1983]. Previous studies have demonstrated that within the order Primates there are phylogenetic differences in the relative proportions of LDH isoenzymes among brain regions [Goodman et al., 1969]. The current study is the first examination of LDH isoenzyme distribution among different primate species at the neuronal subcellular level in neocortical and striatal tissue.

In humans, the adult brain comprises about 2% of total body weight but utilizes about 20% of the energy consumed by an individual [Chugani, 1987; Jacobs et al., 1995]. Prior to adulthood, energy use by the brain is considerably higher, exceeding more than half of the body's total energy budget. Keeping the brain supplied with energy is therefore a critical function. Because the propor-

tion of the body's total energetic budget that is allocated to the brain is so large, evolutionary accommodations to alleviate this systemic energy stress are suggested to have occurred, particularly in human evolution where brain size expansion has been extreme [Aiello and Wheeler, 1995; Aiello and Wells, 2002]. Substantial evolution on the haplorhine lineage has taken place in the electron transport chain in the sequence of protein complexes [Grossman et al., 2004] and in their expression level [Uddin et al., 2004]. Work on neuron-glia metabolic coupling [Pellerin and Magistretti, 2004, 2012; Aubert et al., 2005] has shown that astrocytes use glucose to produce lactate for release to neurons, which in turn use lactate as their preferred substrate. Our previous work showed that the proportion of glial cells relative to neurons increases in haplorhine primates as the overall brain size enlarges, with humans and other apes displaying the highest glia-neuron ratios [Sherwood et al., 2006]. Further significant evolutionary changes have taken place through an increase in the complexity of the synapse in terms of the number of its components, the timing of their expression, and the evolution of individual components [Bayés et al., 2011].

Synaptosomal fractions were analyzed in the current study since there is increasing evidence that lactate is synthesized within astrocytes and the oxidative use of lactate for energy is highly localized in synaptic terminals [O'Brien et al., 2007]. In our research, synaptosomal fractions were isolated from two regions of the primate forebrain, the neocortex and striatum, and compared with total homogenates from these regions. Using Western blot and ELISA techniques, significant phylogenetic and brain region-specific variation in single LDH-B and LDH-A subunits were observed in synaptic protein-enriched fractions. In particular, the relative enrichment of LDH-B in the synaptosome was detected in a manner that was associated with brain size increase across the primate phylogeny.

Differences in the LDH isoenzyme composition of synaptosomes and total homogenates obtained from primate neocortex and striatum were also determined by isoenzyme analysis. The study of LDH heterogeneity in the total homogenate demonstrated that it consists of 5 basic LDH isoenzymes: B4, B3A, A2B2, BA3, and A4. By contrast, in synaptosomal fractions all 5 LDH isoenzymes were detected only in strepsirrhines, NWM, and OWM. In synaptosome-enriched fractions from chimpanzees and humans, LDH-A3B1 was slightly detectable and LDH-A4 was not identified in synaptosomes from the neocortex; in the striatum of chimpanzees and humans

only trace amounts of anaerobic LDH isoforms were detected (fig. 2). Our results are in agreement with previously published results demonstrating selective enrichment of LDH-B in isolated synaptic terminals [McKenna et al., 1993, 1995, 1998].

The most pronounced elevation of LDH-B expression was observed in the synaptosomal fractions of chimpanzee and human neocortex, indicating that during evolution an increased demand for efficient energetic allocation has accompanied modifications of the neocortex to a greater extent than for the striatum. This more intensified energetic cost of neocortical synapses in association with brain expansion, relative to those in the striatum, may be due to the larger average somatic size of neocortical neurons as brains get larger [Sherwood et al., 2003; Kreitzer, 2009], differences in the intrinsic bioenergetic or metabolic properties of cortical and striatal synaptosomes [Choi et al., 2011], or selectively greater volumetric enlargement of the neocortex due to developmental timing [Rapoport, 1990; Finlay and Darlington, 1995]. Further research is needed to characterize the subcellular morphology of neurons in the neocortex and striatum in different primate species that might drive the energetic demand.

We have demonstrated the predominance of aerobic isoforms in chimpanzee and human neocortex, while anaerobic isoforms predominate in strepsirrhine neocortex. This suggests that for haplorhine species synaptosomes utilize aerobic respiration as reported previously [Kauppinen and Nicholls, 1986; Lores-Arnaiz and Bustamante, 2011]. This can mostly be explained by the presence of mitochondria in the synaptosomal fraction. It is well known that mitochondria play a key role in meeting the demands of neuronal synapses for energy (ATP) [Lemire et al., 2008]. This intimate relationship between lactate and the mitochondria, therefore, likely imparts multiple benefits to a high-energy-demanding organ like the brain [Grossman et al., 2001; Herculano-Houzel, 2011].

Different brain region-specific cellular mechanisms could be implicated in the regulation of intracellular LDH-A and LDH-B localization across primate species that can be exerted at any step in the pathway of LDH isoenzyme gene expression or during protein turnover. LDH subunits are encoded by 2 separate genes, LDH-A and LDH-B [Bittar et al., 1996], and they are regulated differently and independently [Bunn and Poyton, 1996; O'Brien et al., 2007]. First, it is possible that relatively larger neurons in the neocortex of primates with larger brains require specific responses to oxygen availability due to their intrinsic bioenergetic properties involved in the propagation of signals across a longer distance [Sherwood et al.,

2006]. In contrast to the LDH-B gene, the LDH-A gene possesses hypoxia recognition sites in its promoter sequence, responsive to a hypoxia-inducible transcription factor (HIF-1) [Bunn and Poyton, 1996] that is known to upregulate LDH-A levels under hypoxic conditions [Semenza et al., 1996]. A second mechanism is LDH-B promoter hypermethylation and the consequences of loss of protein expression. Numerous studies have shown that hypermethylation of normally unmethylated CpG dinucleotides located in a gene promoter is associated with gene silencing at the transcriptional level [Gagneux and Varki, 2001; Das and Singal, 2004]. Preliminary data show differential utilization of conserved CpG methylation of the LDH-B gene between humans and dwarf lemurs [Duka et al., 2010].

Other key components facilitate lactate uptake and utilization by neurons. One is the high-affinity proton-linked monocarboxylic acid transporter (MCT) 2 that is associated with nerve endings [McKenna et al., 1998; Hertz and Dienel, 2005]; it is one of a family of 14 that have different substrate affinities and brain distributions [Brooks, 2002; Pellerin and Magistretti, 2004]. Another member of this transporter family, MCT1, has been shown to exist in a complex with LDH and a chaperone protein, CD147, in association with COX on the mitochondrial inner membrane [Hashimoto et al., 2006]. This finding supports the lactate shuttle and explains the oxidative catabolism of lactate.

LDH has long been known to exhibit different kinetics such that LDH-A more rapidly converts pyruvate to lactate than LDH-B, and LDH-B is more susceptible to inhi-

bition by pyruvate [Dawson et al., 1964]. These properties are important because of the metabolic support they provide for the operation of the lactate shuttle since pyruvate can be converted to acetyl-CoA, via pyruvate dehydrogenase, which then enters the tricarboxylic acid cycle for aerobic respiration. Conversely, the conversion of pyruvate to lactate regenerates NAD⁺ from NADH, which promotes glycolysis. Thus, the distribution of LDH types reported here can be related to metabolic features of their compartments. It is noteworthy that distinct synaptosome subpopulations are differentially susceptible to bioenergetic failure under conditions of increased energy demand [Choi et al., 2009].

In conclusion, LDH isoforms have distinct expression patterns in the neocortex and striatum subcellular fractions of primate species, which are correlated with variation in total brain size. These differences may contribute to divergent lactate dynamics and oxidative capacities in synaptic terminals and may have driven metabolic remodeling from 'anaerobic-glycolytic' to 'aerobic, oxidative phosphorylation-enhanced' metabolism during primate brain evolution.

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References

- Aiello LC, Wells JCK (2002): Energetics and the evolution of the genus *Homo*. *Ann Rev Anthropol* 31:323–338.
- Aiello LC, Wheeler P (1995): The expensive-tissue hypothesis – the brain and the digestive-system in human and primate evolution. *Curr Anthropol* 36:199–221.
- Armstrong E (1983): Relative brain size and metabolism in mammals. *Science* 220:1302–1304.
- Aubert A, Costalat R, Magistretti PJ, Pellerin L (2005): Brain lactate kinetics: modeling evidence for neuronal lactate uptake upon activation. *Proc Natl Acad Sci USA* 102:16448–16645.
- Barrickman NL, Lin MJ (2010): Encephalization, expensive tissues, and energetics: an examination of the relative costs of brain size in strepsirrhines. *Am J Phys Anthropol* 143:579–590.
- Bayés A, van de Lagemaat LN, Collins MO, Croning MD, Whittle IR, Choudhary JS, Grant SG (2011): Characterization of the proteome, diseases and evolution of the human postsynaptic density. *Nat Neurosci* 14:19–21.
- Beebe TJ, Carty DS (1982): Purification and radioimmunoassay of rat lactate dehydrogenase A and B subunits. *Biochem J* 205:313–320.
- Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ (1996): Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. *J Cereb Blood Flow Metab* 16:1079–1089.
- Boddy AM, McGowen MR, Sherwood CC, Grossman LI, Goodman M, Wildman DE (2012): Comparative analysis of encephalization in mammals reveals relaxed constraints on anthropoid primate and cetacean brain scaling. *J Evol Biol* 25:981–994.
- Brooks GA (2002): Lactate shuttles in nature. *Biochem Soc Trans* 30:258–264.
- Bunn HF, Poyton RO (1996): Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 76:839–885.
- Castro MA, Beltrán FA, Brauchi S, Concha II (2009): A metabolic switch in brain: glucose and lactate metabolism modulation by ascorbic acid. *J Neurochem* 110:423–440.
- Charpentier J, Goutefongea R (1964): Electrophoretic characteristics of lactic dehydrogenase in normal and exudative pig muscle. *Nature* 201:1325–1326.
- Choi SW, Gerencser AA, Lee DW, Rajagopalan S, Nicholls DG, Andersen JK, Brand MD (2011): Intrinsic bioenergetic properties and stress sensitivity of dopaminergic synaptosomes. *J Neurosci* 31:4524–4534.

- Choi SW, Gerencsér AA, Nicholls DG (2009): Bioenergetic analysis of isolated cerebrocortical nerve terminals on a microgram scale: spare respiratory capacity and stochastic mitochondrial failure. *J Neurochem* 109:1179–1191.
- Chugani HT (1987): Positron emission tomography: principles and applications in pediatrics. *Mead Johnson Symp Perinat Dev Med* 25:15–18.
- Das PM, Singal R (2004): DNA methylation and cancer. *J Clin Oncol* 22:4632–4642.
- Dawson DM, Goodfriend TL, Kaplan NO (1964): Lactic dehydrogenases: functions of the two types rates of synthesis of the two major forms can be correlated with metabolic differentiation. *Science* 143:929–933.
- Duka T, Papper Z, Stimpson CD, Anderson S, Bonar CJ, Raghanti MA, Wildman DE, Sherwood CC (2010): Phylogenetic Variation in the Methylation of Lactate Dehydrogenase LDHA and LDHB Promoters and Isoenzyme Expression Patterns in the Primate Cerebral Cortex. Abstract Viewer/Itinerary Planner. San Diego, Society for Neuroscience.
- Finlay BL, Darlington RB (1995): Linked regularities in the development and evolution of mammalian brains. *Science* 268:1578–1584.
- Fondy TP, Kaplan NO (1965): Structural and functional properties of the H and M subunits of lactic dehydrogenases. *Ann NY Acad Sci* 119:888–904.
- Gagneux P, Varki A (2001): Genetic differences between humans and great apes. *Mol Phylogenet Evol* 8:2–13.
- Goodman M, Sterner KN (2010): Colloquium paper: phylogenomic evidence of adaptive evolution in the ancestry of humans. *Proc Natl Acad Sci USA* 107:8918–8923.
- Goodman M, Syner FN, Stimson CW, Rankin JJ (1969): Phylogenetic changes in the proportions of two kinds of lactate dehydrogenase in primate brain regions. *Brain Res* 14:447–459.
- Grossman LI, Schmidt TR, Wildman DE, Goodman M (2001): Molecular evolution of aerobic energy metabolism in primates. *Mol Phylogenet Evol* 18:26–36.
- Grossman LI, Wildman DE, Schmidt TR, Goodman M (2004): Accelerated evolution of the electron transport chain in anthropoid primates. *Trends Genet* 20:578–585.
- Hammer Ø, Harper DAT, Ryan PD (2001): Past: paleontological statistics software package for education and data analysis. *Palaentol Electron* 4:1–9.
- Hashimoto T, Hussien R, Brooks GA (2006): Colocalization of MCT1, CD147 and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a mitochondrial lactate. *Am J Physiol Endocrinol Metab* 290:1237–1244.
- Herculano-Houzel S (2011): Scaling of brain metabolism with a fixed energy budget per neuron: implications for neuronal activity, plasticity and evolution. *PLoS One* 6:1–9.
- Hertz L, Dienel GA (2005): Lactate transport and transporters: general principles and functional roles in brain cells. *J Neurosci Res* 79:11–18.
- Hinks M, Masters CJ (1964): Developmental changes in ruminant lactate dehydrogenase. *Biochemistry* 3:1789–1791.
- Hinks M, Masters CJ (1965): The epigenetic control of lactate-dehydrogenase biosynthesis. *Life Sci* 4:679–703.
- Horrobin DF (1998): Schizophrenia: the illness that made us human. *Med Hypotheses* 50:269–288.
- Jacobs B, Chugani HT, Allada V, Chen S, Phelps ME, Pollack DB, Raleigh MJ (1995): Developmental changes in brain metabolism in sedated rhesus macaques and vervet monkeys revealed by positron emission tomography. *Cereb Cortex* 5:222–233.
- Kauppinen RA, Nicholls DG (1986): Synaptosomal bioenergetics: the role of glycolysis, pyruvate oxidation and responses to hypoglycaemia. *Eur J Biochem* 158:159–165.
- Koen AL, Goodman M (1969): Lactate dehydrogenase isozymes: qualitative and quantitative changes during primate evolution. *Biochem Genet* 3:457–447.
- Koen AL, Goodman M (1971): Metabolic immaturity in mental retardation: quantitative relationships of lactate dehydrogenase isozymes. *J Ment Defic Res* 15:229–235.
- Kreitzer AC (2009): Physiology and pharmacology of striatal neurons. *Annu Rev Neurosci* 32:127–147.
- Latner AL, Skillen AW (1964): Lactate dehydrogenase isoenzymes in foetal and neonatal tissues. *J Embryol Exp Morphol* 12:501–509.
- Laughton JD, Charnay Y, Belloir B, Pellerin L, Magistretti PJ, Bouras C (2000): Differential messenger RNA distribution of lactate dehydrogenase LDH-1 and LDH-5 isoforms in the rat brain. *Neuroscience* 96:619–625.
- Leiblich A, Cross SS, Catto JW, Phillips JT, Leung HY, Hamdy FC, Rehman I (2006): Lactate dehydrogenase-B is silenced by promoter hypermethylation in human prostate cancer. *Oncogene* 25:2953–2960.
- Lemire J, Mailloux RJ, Appanna VD (2008): Mitochondrial lactate dehydrogenase is involved in oxidative-energy metabolism in human astrocytoma cells (CCF-STTG1). *PLoS One* 3:e1550.
- Lores-Arnaiz S, Bustamante J (2011): Age-related alterations in mitochondrial physiological parameters and nitric oxide production in synaptic and non-synaptic brain cortex mitochondria. *Neuroscience* 188:117–124.
- Lowenthal A, Van Sande M, Karcher D (1961): Heterogeneity of lactic and malic dehydrogenase in serum, cerebrospinal fluid, and brain extracts in man and sheep. *Ann NY Acad Sci* 94:988–995.
- McKenna MC, Tildon JT, Stevenson JH, Boatright R, Huang S (1993): Regulation of energy metabolism in synaptic terminals and cultured rat brain astrocytes: differences revealed using aminooxyacetate. *Dev Neurosci* 15:320–329.
- McKenna MC, Tildon JT, Stevenson JH, Huang X, Kingwell KG (1995): Regulation of mitochondrial and cytosolic malic enzymes from cultured rat brain astrocytes. *Neurochem Res* 20:1419–1501.
- McKenna MC, Tildon JT, Stevenson JH, Hopkins IB, Huang X, Couto R (1998): Lactate transport by cortical synaptosomes from adult rat brain: characterization of kinetics and inhibitor specificity. *Dev Neurosci* 20:300–309.
- Milne EM, Doxey DL (1987): Lactate dehydrogenase and its isoenzymes in the tissues and sera of clinically normal dogs. *Res Vet Sci* 43:222–224.
- Navarrete A, van Schaik CP, Isler K (2011): Energetics and the evolution of human brain size. *Nature* 480:91–93.
- Nisselbaum JS, Bodansky O (1959): Reactions of lactic dehydrogenase from various rabbit organs with antirabbit muscle lactic dehydrogenase. *J Biol Chem* 234:3276–3280.
- Nisselbaum JS, Bodansky O (1961): Purification and properties of human heart lactic dehydrogenase. *J Biol Chem* 236:323–327.
- O'Brien J, Kla KM, Hopkins IB, Malecki EA, McKenna MC (2007): Kinetic parameters and lactate dehydrogenase isozyme activities support possible lactate utilization by neurons. *Neurochemical Res* 32:597–607.
- Pellerin L (2003): Lactate as a pivotal element in neuron-glia metabolic cooperation. *Neurochem Int* 43:331–338.
- Pellerin L, Magistretti PJ (2004): Neuroenergetics: calling upon astrocytes to satisfy hungry neurons. *Neuroscientist* 10:53–62.
- Pellerin L, Magistretti PJ (2012): Sweet sixteen for ANLS. *J Cereb Blood Flow Metab* 32:1152–1166.
- Plagemann PG, Gregory KF, Wroblewski F (1960): The electrophoretically distinct forms of mammalian lactic dehydrogenase. *J Biol Chem* 235:2282–2287.
- Rapoport SI (1990): Integrated phylogeny of the primate brain, with special reference to humans and their diseases. *Brain Res Brain Res Rev* 15:267–294.
- Ross JM, Öberg J, Brené S, Coppotelli G, Terzoglou M, Pernold K, Gojny M, Sitnikov R, Kehr J, Trifunovic A, Larsson NG, Hoffer BJ, Olson L (2010): High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *Proc Natl Acad Sci USA* 107:20087–20092.
- Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, Giallongo A (1996): Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* 271:32529–32537.
- Sherwood CC, Duka T, Stimpson CD, Schenker NM, Garrison AR, Schapiro SJ, Baze WB, McArthur MJ, Erwin JM, Hof PR, Hopkins WD (2010): Neocortical synaptophysin asymmetry and behavioral lateralization in chimpanzees (*Pan troglodytes*). *Eur J Neurosci* 31:1456–1464.

- Sherwood CC, Lee PW, Rivara CB, Holloway RL, Gilissen EP, Simmons RM, Hakeem A, Allman JM, Erwin JM, Hof PR (2003): Evolution of specialized pyramidal neurons in primate visual and motor cortex. *Brain Behav Evol* 61: 28–44.
- Sherwood CC, Stimpson CD, Raghanti MA, Wildman DE, Uddin M, Grossman LI, Goodman M, Redmond JC, Bonar CJ, Erwin JM, Hof PR (2006): Evolution of increased glia-neuron ratios in the human frontal cortex. *Proc Natl Acad Sci USA* 103:13606–13611.
- Syner FN, Goodman M (1966): Differences in the lactic dehydrogenases of primate brains. *Nature* 209:426–428.
- Uddin M, Opazo JC, Wildman DE, Sherwood CC, Hof PR, Goodman M, Grossman LI (2008): Molecular evolution of the cytochrome C oxidase subunit 5A gene in primates. *BMC Evol Biol* 8:8.
- Uddin M, Wildman DE, Liu G, Xu W, Johnson RM, Hof PR, Kapatos G, Grossman LI, Goodman M (2004): Sister grouping of chimpanzees and humans as revealed by genome-wide phylogenetic analysis of brain gene expression profiles. *Proc Natl Acad Sci USA* 101: 2957–2962.
- Vesell ES (1961): Significance of the heterogeneity of lactic dehydrogenase activity in human tissues. *Ann NY Acad Sci* 94:877–889.
- Vesell WS, Bearn G (1961): Isozymes of lactic dehydrogenase in human tissues. *J Clin Invest* 40:586–591.