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In vivo HMRS and lipidomic profiling reveals comprehensive changes of hippocampal metabolism during aging in mice

Lejun Lin [a, 1], Bofeng Cao [b, 1], Zhiying Xu [a, **], Yanbin Sui [b], Jiao Chen [b], Qiang Luan [c], Ruifang Yang [d], Shanchun Li [a], Ke Feng Li [e, *]

[a] Department of Nuclear Medicine, Yantai Yuhuangding Hospital, Yantai, 264000, Shandong Province, China
[b] Medical Imaging Center, Yantai Yuhuangding Hospital, Yantai, 264000, Shandong Province, China
[c] Muping Hospital of Traditional Chinese Medicine, Yantai, 264100, Shandong Province, China
[d] Department of Clinical Laboratory, The Affiliated Hospital of Binzhou Medical University, Yantai, 256603, Shandong Province, China
[e] Tianjin SunnyPeak Biotech Co., Ltd, Tianjin, 300057, China

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ABSTRACT

Aging is characterized by various cellular changes in the brain. Hippocampus is important for systemic aging and lifespan control. There is still a lack of comprehensive overview of metabolic changes in hippocampus during aging. In this study, we first created an accelerated brain aging mice model through the chronic administration of D-galactose. We then performed a multiplatform metabolomic profiling of mice hippocampus using the combination of in vivo 9.4 T HMRS and in vitro LC-MS/MS based lipidomics. We found N-acetylaspartic acid (NAA), gama-aminobutyric acid (GABA), glutamate/glutamine, taurine, choline, sphingolipids (SMs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs), phosphatidylglycerols (PGs) and phosphatidylserines (PSs), all of them decreasing with the aging process in mice hippocampus. The changes of sphingolipids and phospholipids were not limited to one single class or molecular species. In contrast, we found the significant accumulation of lactate, myoinositol and phosphatidylcholines (PCs) along with aging in hippocampus. SM (d18:1/20:2), PE (36:2), PG (34:1), PI (36:4), PS (18:0/20:4) and PC (36:0) have the most significant changes along with aging. Network analysis revealed the striking loss of biochemical connectivity and interactions between hippocampal metabolites with aging. The correlation pattern between metabolites in hippocampus could function as biomarkers for aging or diagnosis of aging-related diseases.

1. Introduction

Aging is a complicated multifactorial process characterized by the gradual and overall loss of various physiological functions. Brain aging is an important aspect of aging process and oxidative stress has been proposed to be a major cause in brain aging and age-related diseases such as Alzheimer's disease and Parkinson's diseases [1]. D-galactose is a reducing sugar that can be oxidized into hydrogen peroxide (H_2O_2) when its concentration is greater than the normal level and thus induces oxidative stress. In addition, D-galactose can form advanced glycation end products (AGEs) which aggravate oxidative stress in vivo [2]. Chronic administration of D-galactose to mice mimics many features of natural brain aging and has become a widely used animal mode for the study of mechanisms and screening of drugs for brain aging [3].

The aging process profoundly impacts the brain and in many cases, age-related differences are confined to specific regions of the brain, in particular the hippocampus and the frontal and parietal lobes [4]. Hippocampus is the key region that is crucial for cognitive functions, memory and spatial navigation. Altered metabolic profiles in hippocampus during aging have been reported in human [5–7] and mice [8–10] using in vivo HMRS with inconsistent results which might be due to the low-magnetic field used in their studies. In addition, most of the studies focused on the prominent resonances in HMRS spectra rather than determining an extensive neurochemical profile. Moreover, little information is known related to the molecular species and changes of phospholipids and sphingolipids in mice hippocampus during aging.

* Corresponding author.
** Corresponding author.
E-mail addresses: easy0603@163.com (Z. Xu), kefengl@sunnypeak.org (K.F. Li).
[1] Lejun Lin and Bofeng Cao contribute equally to this work and are co-first authors.

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Higher field strength HMRS provides increased signal-to-noise and spectral dispersion which are sufficient for accurate measurement of more than 20 neurochemicals in mice hippocampus except lipids. It was used for in vitro measurement of neurochemical changes in age-related Alzheimer's disease with complicated extraction procedures in a recent study [11]. No reports are available for direct characterization of hippocampal metabolic changes during brain aging in vivo using 9.4T HMRS without time-consuming metabolites extraction step. In order to obtain a comprehensive metabolic profile, we also modified a previous method and developed a targeted lipidomic approach based on LC-MS/MS for simultaneous quantification of major lipids in mice hippocampus including phospholipids, sphingolipids and ceramides [12].

In this study, an accelerated aging mice model was created to mimic the natural brain aging through the chronic administration of D-galactose. We performed a comprehensive metabolic profiling of mice hippocampus during aging using two complementary metabolomic platforms. A total of 164 endogenous metabolites in mice hippocampus were targeted including neurotransmitters, amino acids, phospholipids, sphingolipids (SMs) and ceramides. We also performed metabolic network analysis to explore the interactions of hippocampal metabolites with aging. Our data provided a footprint of comprehensive metabolic aging in mice hippocampus.

2. Methods and materials

2.1. Animals and treatment

Five-week-old male C57BL/6J mice were obtained from Peking Union Medical College. All animal experiments were performed according to the guidelines approved by the Ethical Committee of Yantai Yuhuangding Hospital, China. Animals were housed in a temperature (22–25 °C) and humidity (40%–50%) controlled vivarium on a 12 h dark/12 h light schedule. All mice were free access to food and water.

Beginning at 8 weeks of age, animals were injected intraperitoneally (i.p.) daily with 120 mg/kg of D-galactose or vehicles (0.9% physiological saline, 210 mg/kg, ip) for 60 days, respectively. Morris water maze test and in vivo HMRS were performed on 30 d and 60 d. After the behavior test and in vivo HMRS, the mice were sacrificed and the hippocampi were immediately dissected and frozen in liquid nitrogen for H&E staining and lipidomic profiling.

2.2. Behavior test

Morris water maze (MWM) test was used to evaluate the hippocampal-dependent spatial learning and memory impairment induced by D-galactose. The test was performed according to the protocol described by Christine et al. [13]. The time and the distance of swimming for each mouse spent were recorded with a computerized video system.

2.3. In vivo ^{1}HMRS measurements of neurochemicals in mice hippocampus

For imaging, the animals were anesthetized using a gas mixture of air:oxygen (1:1) with 1–1.5% isoflurane and allowed to breathe spontaneously. Body temperature was kept within 36.5–37.5 °C during HMRS scanning. ^{1}HMRS acquisitions were performed on a 9.4T/20 MRI instrument (Bruker, Avance 400 MHz). The in vivo 1H MRS spectra were acquired from the voxels located in the left hippocampus using PRESS sequence [14]. The voxel size of the hippocampal VOI was 2.2 × 1.2 × 2.4 mm^3; and hippocampal tissues were estimated to be over 80% in a given VOI. The following MRS parameters were set up: TR = 4000 ms, TE = 12 ms, NA = 512, spectral width = 8012 Hz, number of acquired complex points = 2048 yielding a spectral resolution of 3.91 Hz/pts. Concentrations of neurochemicals were quantified using an MRS data analysis package and LCModel (linear combination of model spectra of metabolite solutions) software [15].

2.4. Histopathology

Hematoxylin and eosin (HE) staining was performed using the standard histological technique. Briefly, the left hippocampus tissues were sliced and fixed in 4% paraformaldehyde in 0.01 M PBS (pH 7.4) for 8 h, dehydrated, and embedded with paraffin. Sections were cut into 4 mm thick pieces, deparaffinized, and rehydrated in ethanol and distilled water. The slices were then stained with HE and observed under light microscopy.

2.5. Metabolomic profiling of lipids in hippocampus using LC-MS/MS

About 50 mg of left hippocampus were sliced and homogenized in 1.5 ml of prechilled extract buffer containing methanol-acetonitrile-acetone-H_2O (30:30:30:10) and ^{13}C labeled isotope standards [16]. The mixture was centrifuged at 16,000 g for 10 min and the supernatant was taken for lipidomic profiling using LC-MS/MS.

LC–MS/MS analysis was conducted using a UHPLC system (UHPLC1260, Agilent, USA) coupled to a Qtrap 4500 hybrid triple quadrupole mass spectrometer (AB SCIEX, USA) operated in multiple reaction monitoring (MRM) mode. The dwell time was set at 10 ms. Each sample was injected twice, one for negative (ESI−) mode scanning and the other one for positive (ESI+) scanning. The MRMs were optimized using purified standards. A total of 152 lipids including ceramides, sphingolipids and phospholipids were targeted. The source conditions were as follows: electrospray voltage of −4500 V on negative mode and 5500 V on positive mode, source temperature of 450 °C, curtain gas of 30, CAD gas of 11, gas 1 and gas 2 of 30 and 30 psi, respectively.

The chromatographic conditions were adapted from the previous work with modifications [16]. Briefly, the separation was carried out on an ACQUITY UPLC BEH C18 column (2.1 × 150 mm, 1.7 μm). Mobile phase A was 95% H_2O with 20 mM ammonium formate and 5% acetonitrile (pH 4). Mobile phase.

B was 100% acetonitrile. The gradient was follows: 95% A, 3 min-95% A, 3.1 min-85% A, 6.0 min-85% A, 6.1 min- 75% A, 10 min-75% A, 15 min-0% A, 25 min-0%, 26 min-95% A, and 31 min-end. The flow rate was 300 μl/min.

2.6. Statistical analysis

Unpaired t test was performed to compare the neurochemical changes between control and galactose treatment group. Data were expressed as mean ± SD. The significance of the differences was indicated as $^{*}P < 0.05$. Before statistical analysis, AUC for each metabolite was normalized and log 2 transformed. Partial least squares discriminant analysis (PLS-DA), VIP analysis, heatmap analysis and Pearson correlation analysis were analyzed using metaboanalyst (http://www.metaboanalyst.ca).

3. Results

3.1. Confirmation of aging model

In this study, we found that compared with the saline-injected group, chronic administration of D-galactose led to the symptoms

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which were similar to natural aging such as dried and dull hair, flabby skin and slow response. In order to verify the success of aging model, we performed Morris water Maze behavior test. Our results showed that D-galactose treatment resulted in the significant prolongation of mean escape latencies ($p < 0.05$) (Supplemental Fig. 1). This suggested the impairment of spatial learning and memory in mice treated with D-galactose which is also the feature of natural brain aging and aging-related diseases.

The morphological changes of hippocampal neurons during aging were visualized after HE staining. The hippocampal neuron cells were densely, closely and regularly arranged in the control group (Supplemental Fig. 2a). Progressive neuro cells loss was observed at 30 d after D-galactose treatment. The arrangement of neuron cells was loose and degenerative neurons cells appeared (Supplemental Fig. 2b). The density of the neuro cells decreased remarkably and the number of degenerative neurons cells increased dramatically at 60 d (Supplemental Fig. 2c). All these behavior and neuro cell morphological features are similar to those in human natural aging and aging-related diseases. These data indicated that D-galactose induced accelerated aging mice model was successfully created.

3.2. Quantitative LCModel ^{1}H-MRS analysis

To explore the possible age-associated metabolic changes in hippocampus, we performed in vivo HMRS to quantify the essential neurochemicals in hippocampus. The representative ^{1}H NMR spectra acquired in the hippocampus of mice with and without galactose treatment at 30 and 60 d was showed in Supplemental Fig. 3. LC Model simulation allows the accurate quantification of eight metabolites in mice hippocampus (Fig. 1). Our results revealed the significant decrease of neurotransmitters in hippocampus in response to D-galactose treatment including N-acetylaspartic acid (NAA), gama-aminobutyric acid (GABA) and glutamate (Fig. 1). Compared with the concentration of choline (0.28 ± 0.062 mM) in saline treatment group, the mean concentration of choline in model group tended to be reduced at day 30 and reached significance on day 60 d (0.16 ± 0.074 mM). Sulfur amino acid taurine has a general trend of decrease during aging and was only about 40% of the control group treated with saline at 60 d. However, myo-inositol and lactate accumulated during aging and their concentrations in the hippocampus of mice treated with D-galactose were significantly higher than those in control group at 60 d. Total creatinine levels in mice hippocampus were not changed in model group compared with the controls. Lipids were detectable at 1.25–1.30 ppm on ^{1}HMRS (Supplemental Fig. 3, Peak 1). Total lipids decreased significantly with the progressive administration of D-galactose.

3.3. Changes of lipids profile in hippocampus revealed by LC-MS/MS based lipidomics

To further investigate the changes of lipids during aging, we performed targeted lipidomics to quantify the major lipids in

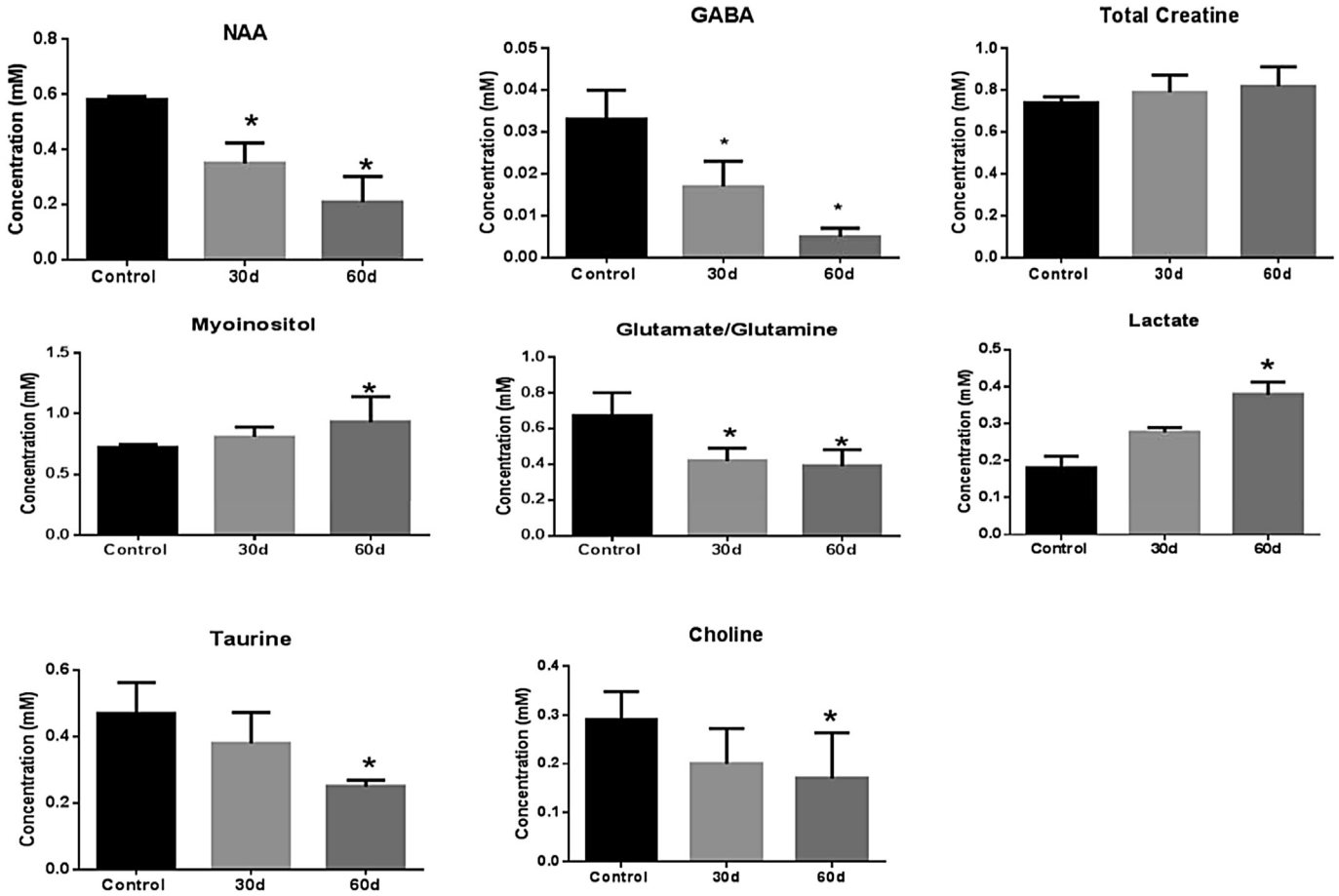

Fig. 1. The changes of neurochemical metabolites in hippocampus during aging measured by in vivo 1HMRS. Data was mean ± SD (n = 6). *P < 0.05 versus control group. NAA: N-acetylaspartate, GABA: γ-aminobutyric acid.

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hippocampus including PCs, PEs, PGs, PIs, SMs and ceramides. A color heatmap in Supplemental Fig. 4 showed the relative abundance of all lipids measured in this study. Further PLS-DA analysis revealed the dramatic changes in lipids metabolism of mice hippocampus during aging (Fig. 2). VIP analysis was performed followed by PLS-DA and the top 15 most discriminating lipids were listed in Fig. 3. There was a general increase in PCs in mice hippocampus during aging. In contrast, the major PE, PI and PG lipids in hippocampus decreased along with the administration of D-galactose such as PE (36:2), PE (34:1), PE (34:2), PG (34:1) and PI (36:4). In addition, there was also a significant reduction on SMs. SM (d18:1/20:2) has the most significant decrease in SMs and was only 30% of the control groups at 60 d. Ceramides showed a trend of increase in response to D-galactose treatment but the changes were not statistically significant among the three groups (Data was not shown).

3.4. The gradual loss of correlations between hippocampal metabolites during aging

We performed network analysis using Person correlation to investigate the interactions of hippocampal metabolites during aging. Surprisingly, we discovered a striking and distinct correlation pattern associated with hippocampal metabolites in response to aging (Supplemental Fig. 5A–C). In control group, the metabolites in mice hippocampus were tightly correlated (Fig. 4A). A total of 7644 significantly positive correlations (Pearson coefficient >0.8) and 4938 negative correlations (Pearson coefficient <−0.8) were observed in correlation matrix of the control group (Supplemental Fig. 5D). The number of both negative and positive correlations between hippocampal metabolites in mice decreased dramatically during aging (Supplemental Fig. 5B and C). At 60 d, only 1762 of positive correlations (Pearson coefficient >0.8) and 1164 of negative correlations (Pearson coefficient <−0.8) were found in mice hippocampus (Fig. 4D).

We then analyzed the metabolites which are significantly correlated with the changes of hippocampal neurotransmitters during aging. The top 25 metabolites significantly correlated with the neurotransmitters were listed in Fig. 4. We showed that the changes of GABA during aging in mice hippocampus were positively correlated with glutamate/glutamine, NAA, sphingolipids, PI lipids, PE lipids and choline, while negatively correlated with PC lipids, myoinositol and lactate. Glutamate/glutamate is also positively correlated with ceramides and taurine.

4. Discussion

Hippocampus is known to control important functions such as memories. A recent study showed that hippocampus also has a programmatic role in aging development through immune–neuroendocrine integration, immune inhibition or GnRH restoration [17]. In this study, using the combination of in vivo HMRS and LC-MS/MS targeted lipidomics, we showed the dramatic metabolic changes in mice hippocampus including neurotransmitters, amino acids, organic acids and lipids during aging process.

Our data supported the notion that neurotransmitters tend to decline with age. Glutamate is the most abundant excitatory neurotransmitter and associated with learning and memory in the brain [18]. Studies have shown older subjects to have lower glutamate concentration in the motor cortex, parietal gray matter, basal ganglia and the frontal white matter [19,20]. However, the changes of glutamate in hippocampus was not fully explored with inconsistent results, which are probably due to the limitation of techniques [21–23]. Our data with improved accuracy of HMRS at 9.4 T revealed a significant reduction of glutamate concentration in mice hippocampus with age. Although the decrease of certain inhibitory neurotransmitters has been reported in aging associated Alzheimer's disease [10], our findings provide the evidence that natural aging entails the significant reduction of inhibitory neurotransmitters including GABA and taurine.

We found the accumulation of lactate and myo-inositol in mice hippocampus during aging which might be used as predictive biomarkers for monitoring aging process. The increase of lactate in

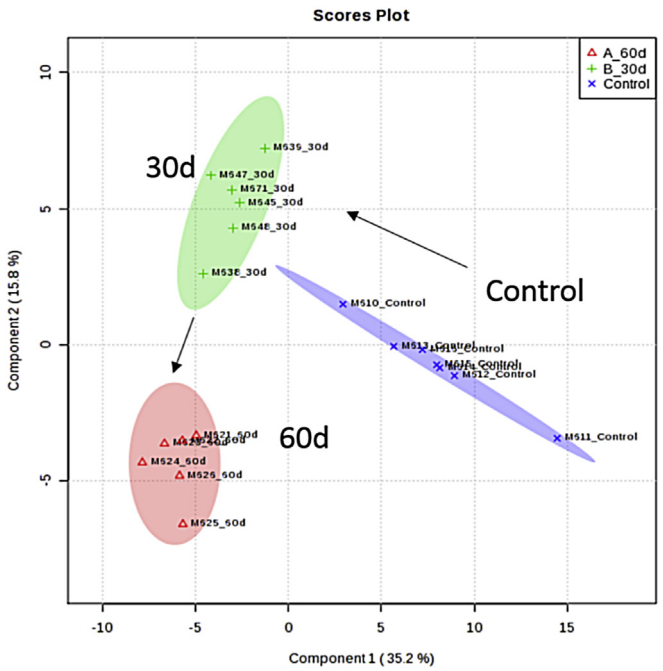

Fig. 2. PLS-DA analysis revealed dramatic changes on the lipids metabolism in mice hippocampus during aging. The major lipids in mice hippocampus were measured by LC-MS/MS and analyzed by partial least square analysis (PLS-DA). N = 6 per group.

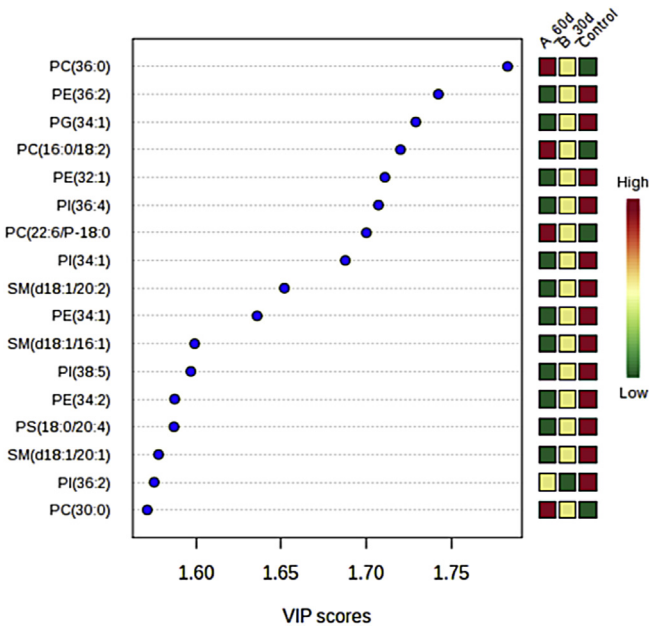

Fig. 3. The top 15 most discriminating lipids in mice hippocampus in response to the progressive administration of D-galactose. The metabolites were ranked by variable importance in projection (VIP) scores.

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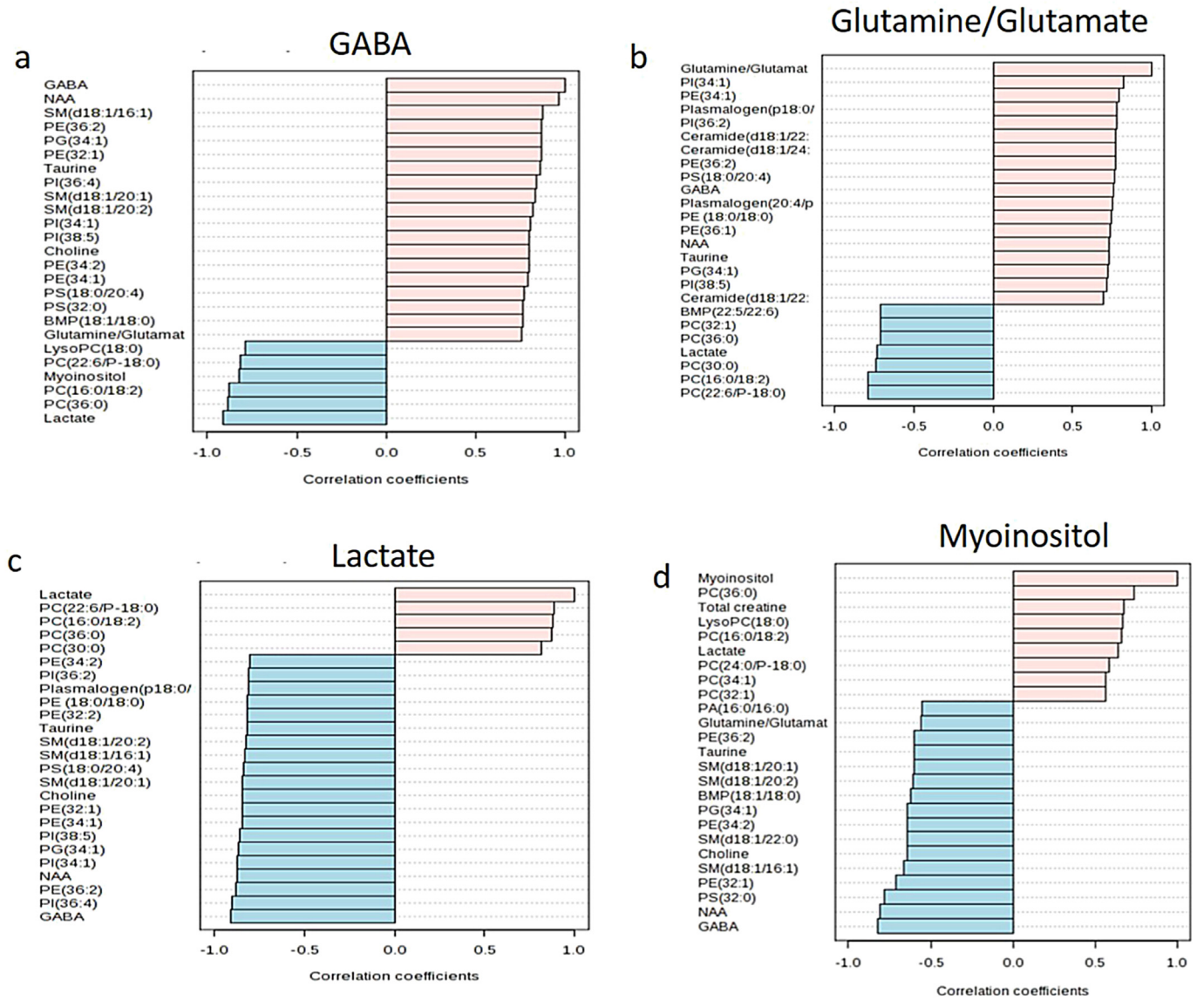

Fig. 4. The top 25 metabolites correlated with the changes of neurotransmitters during aging. A: GABA; B: Glutamine/Glutamate; C: Lactate; D: Myoinositol. The AUCs of each metabolite was normalized and log transformed. Pearson correlation was conducted in metaboanalyst.

mice hippocampus was likely due to the increased LDH-A/LDH-B ratio [11]. An increase in hippocampal myo-inositol had been reported in aged rats using in vitro HMRS [24]. The present study using in vivo HRMS thus added the knowledge of metabolic changes in mice hippocampus. An increased myo-inositol concentration in aged animals might be due to the increased number of astrocytes [24].

In addition, our metabolomic study revealed the down-regulation of sphingolipid metabolism in mice hippocampus with aging process. Sphingomyelins are the dominant sphingolipids in lipid raft domains of mammalian cell membrane and play important roles in regulating a variety of physiological processes such as apoptosis, cell growth arrest and differentiation [25]. The decrease of SMs might be linked to membrane-associated oxidative stress with aging process [26]. In terms of molecular species of sphingolipids, SM (d18:1/20:2) has the most profound changes in hippocampus. The perturbed of sphingolipid metabolism might result in the progressive degradation of neuro cells.

Interestingly, our metabolomics analysis of mice hippocampus showed significant changes of many phospholipids during brain aging. The changes were not limited to any single class of phospholipid. Rather, the age-related changes in mice hippocampus occurred in a wide range of phospholipids including PCs, PEs, PIs, PGs and PSs. Since phospholipids are the main components of neuronal membrane, the changes of their composition influence the membrane function and properties which in turn strongly modulate the cellular processes [26]. PC (36:0) is the most discriminant metabolite with about 20% increase in mice hippocampus at 60 d. The role of this particular molecular phospholipid is largely unknown and might be related to membrane fluidity [27]. Increase of PC (36:0) might reduce the membrane fluidity in mice during aging. A recent study demonstrated the decrease of PE lipids in human hippocampus during aging [28]. Consistent with these data, we found lower levels of PE lipids in mice hippocampus along with aging. The age-related decrease of PGs, PSs and PIs has not been found by previous studies in mice hippocampus.

Our network analysis suggested the dramatic loss of the interlinks between hippocampal metabolites during aging. The

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complex interactions between metabolites are regulated through metabolic networks. Some of the metabolic interactions between neurotransmitters and PI lipids have been reported as regulators of neurotransmitter signaling in neurological and psychiatric diseases [29]. Besides, PI lipids, here, we also showed that neurotransmitter GABA and glutamate/glutamine were negatively correlated with PC lipids. The molecular mechanisms regarding to the interactions between PC lipids and neurotransmitters have not been fully explored and probably are related to the membrane dynamics on synapses [30]. More important, the change of the pattern of biochemical connectivity between metabolites in hippocampus might be the key feature of aging and could be served as biomarkers for aging and aging-related diseases.

Conflict of interest

The authors declare no competing financial interest.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.12.009.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.12.009.

References

[1] M.T. Lin, M.F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases, Nature 443 (2006) 787–795.
[2] Y.Y. Liu, B.V. Nagpure, P.T. Wong, J.S. Bian, Hydrogen sulfide protects SH-SY5Y neuronal cells against d-galactose induced cell injury by suppression of advanced glycation end products formation and oxidative stress, Neurochem. Int. 62 (2013) 603–609.
[3] K. Parameshwaran, M.H. Irwin, K. Steliou, C.A. Pinkert, D-galactose effectiveness in modeling aging and therapeutic antioxidant treatment in mice, Rejuvenation Res. 13 (2010) 729–735.
[4] R. Peters, Ageing and the brain, Postgrad. Med. J. 82 (2006) 84–88.
[5] N. Schuff, F. Ezekiel, A.C. Gamst, D.L. Amend, A.A. Capizzano, A.A. Maudsley, M.W. Weiner, Region and tissue differences of metabolites in normally aged brain using multislice 1H magnetic resonance spectroscopic imaging, Magn. Reson. Med. 45 (2001) 899–907.
[6] S. Gruber, K. Pinker, F. Riederer, M. Chmelík, A. Stadlbauer, M. Bittsanský, V. Mlynárik, R. Frey, W. Serles, O. Bodamer, E. Moser, Metabolic changes in the normal ageing brain: consistent findings from short and long echo time proton spectroscopy, Eur. J. Radiol. 68 (2008) 320–327.
[7] P.K. Mandal, H. Akolkar, M. Tripathi, Mapping of hippocampal pH and neurochemicals from in vivo multi-voxel 31P study in healthy normal young male/female, mild cognitive impairment, and Alzheimer's disease, J. Alzheimers Dis. (2012) S75–S86. Suppl. 3.
[8] J.M. Duarte, K.Q. Do, R. Gruetter, Longitudinal neurochemical modifications in the aging mouse brain measured in vivo by 1H magnetic resonance spectroscopy, Neurobiol. Aging 35 (2014) 1660–1668.
[9] S.Q. Chen, Q. Cai, Y.Y. Shen, P.J. Wang, G.J. Teng, W. Zhang, F.C. Zang, Age-related changes in brain metabolites and cognitive function in APP/PS1 transgenic mice, Behav. Brain Res. 235 (2012) 1–6.
[10] I.Y. Choi, P. Lee, W.T. Wang, D. Hui, X. Wang, W.M. Brooks, E.K. Michaelis, Metabolism changes during aging in the hippocampus and striatum of glud1 (glutamate dehydrogenase 1) transgenic mice, Neurochem. Res. 39 (2014) 446–455.
[11] Y. Lin, J. Yao, Y. Chen, L. Pang, H. Li, Z. Cao, K. You, H. Dai, R. Wu, Hippocampal neurochemical changes in senescent mice induced with chronic injection of D-galactose and $NaNO_2$: an in vitro high-resolution NMR spectroscopy study at 9.4T, PLoS One 9 (2014) e88562.
[12] K. Li, X. Wang, V.R. Pidatala, C.P. Chang, X. Cao, Novel quantitative metabolomic approach for the study of stress responses of plant root metabolism, J. Proteome Res. 13 (2014) 5879–5887.
[13] M.W. Christine, B. Victoria, R.P. Gina, Spatial and reversal learning in the Morris water maze are largely resistant to six hours of REM sleep deprivation following training, Learn. Mem. 18 (2011) 422–434.
[14] J.H. Park, H. Lee, R. Makaryus, M. Yu, S.D. Smith, K. Sayed, T. Feng, E. Holland, A. Van der Linden, T.G. Bolwig, G. Enikolopov, H. Benveniste, Metabolic profiling of dividing cells in live rodent brain by proton magnetic resonance spectroscopy (1HMRS) and LC model analysis, PLoS One 12 (2014) e94755.
[15] S.W. Provencher, Estimation of metabolite concentrations from localized in vivo proton NMR spectra, Magn. Reson. Med. 30 (1993) 672–679.
[16] E.J. Want, P. Masson, F. Michopoulos, I.D. Wilson, G. Theodoridis, R.S. Plumb, Global metabolic profiling of animal and human tissues via UPLC-MS, Nat. Protoc. 8 (2013) 17–32.
[17] G. Zhang, J. Li, S. Purkayastha, Y. Tang, H. Zhang, Y. Yin, B. Li, G. Liu, D. Cai, Hypothalamic programming of systemic ageing involving IKK-β, NF-κB and GnRH, Nature 9 (2013) 211–216.
[18] A.C. Fontana, Current approaches to enhance glutamate transporter function and expression, J. Neurochem. (2015), http://dx.doi.org/10.1111/jnc.13200.
[19] L. Chang, C.S. Jiang, T. Ernst, Effects of age and sex on brain glutamate and other metabolites, Magn. Reson. Imaging 27 (2009) 142–145.
[20] L.G. Kaiser, N. Schuff, N. Cashdollar, M.W. Weiner, Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T, Neurobiol. Aging 26 (2005) 665–672.
[21] M. Banay-Schwartz, A. Lajtha, M. Palkovits, Changes with aging in the levels of amino acids in rat CNS structural elements. 1. Glutamate and related amino acids, Neurochem. Res. 14 (1989) 555–562.
[22] R.J. Dawson, D.R. Wallace, M.J. Meldrum, Endogenous glutamate release from frontal cortex of adult and aged rats, Neurobiol. Aging 10 (1989) 665–668.
[23] J.M. Ross, J. Öberg, S. Brené, G. Coppotelli, M. Terzioglu, K. Pernold, M. Goiny, High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20087–20092.
[24] V. Paban, F. Fauvelle, B. Alescio-Lautier, Age-related changes in metabolic profiles of rat hippocampus and cortices, Eur. J. Neurosci. 31 (2010) 1063–1073.
[25] Y.A. Hannun, L.M. Obeid, Principles of bioactive lipid signaling: lessons from sphingolipids, Nat. Rev. Mol. Cell Biol. 9 (2008) 139–150.
[26] R.G. Cutler, J. Kelly, K. Storie, W.A. Pedersen, A. Tammara, K. Hatanpaa, J.C. Troncoso, M.P. Mattson, Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 2070–2075.
[27] C. Klose, M.A. Surma, K. Simons, Organellar lipidomics-background and perspectives, Curr. Opin. Cell Biol. 25 (2013) 406–413.
[28] S.E. Hancock, M.G. Friedrich, T.W. Mitchell, R.J. Truscott, P.L. Else, Decreases in phospholipids containing adrenic and arachidonic acids occur in the human hippocampus over the adult lifespan, Lipids 50 (2015) 861–872.
[29] J.A. Allen, R.A. Halverson-Tamboli, M.M. Rasenick, Lipid raft microdomains and neurotransmitter signalling, Nat. Rev. Neurosci. 8 (2007) 128–140.
[30] V. García-Morales, F. Montero, D. González-Forero, G. Rodríguez-Bey, L. Gómez-Pérez, M. Medialdea-Wandosse, G. Domínguez-Vías, J.M. García-Verdugo, B. Moreno-López, Membrane-derived phospholipids control synaptic neurotransmission and plasticity, PLoS Biol. 13 (2015) e1002153.

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