Lower brain pH as a shared endophenotype of psychotic disorders

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34

35 **Running title**

36 Lower brain pH in psychotic disorders

37 Abstract

38 Lower pH is a well-replicated finding in the postmortem brains of patients with schizophrenia and
 39 bipolar disorder. Interpretation of the data, however, is controversial as to whether this finding
 40 reflects a primary feature of the diseases or is a result of confounding factors such as medication,
 41 postmortem interval, and agonal state. To date, systematic investigation of brain pH has not been
 42 undertaken using animal models, which can be studied without confounds inherent in human studies.
 43 In the present study, we first confirmed that the brains of patients with schizophrenia and bipolar
 44 disorder exhibit lower pH values by conducting a meta-analysis of existing datasets. We then
 45 utilized neurodevelopmental mouse models of psychiatric disorders in order to test the hypothesis
 46 that lower brain pH exists in these brains compared to controls due to the underlying
 47 pathophysiology of the disorders. We measured pH, lactate levels, and related metabolite levels in
 48 brain homogenates from three mouse models of schizophrenia (*Schnurri-2* KO, forebrain-specific
 49 *calcineurin* KO, and *neurogranin* KO mice) and one of bipolar disorder (*Camk2a* HKO mice), and
 50 one of autism spectrum disorders (*Chd8* HKO mice). All mice were drug-naïve with the same
 51 postmortem interval and agonal state at death. Upon postmortem examination, we observed
 52 significantly lower pH and higher lactate levels in the brains of model mice relative to controls.
 53 There was a significant negative correlation between pH and lactate levels. These results suggest
 54 that lower pH associated with increased lactate levels is a pathophysiology of such diseases rather
 55 than mere artifacts.

56

57 Introduction

58 Schizophrenia, bipolar disorder, and autism spectrum disorders (ASD) are highly heritable
 59 psychiatric conditions, with clinical features transcending diagnostic categories^{1,2}. Accumulating
 60 evidence indicates that some genetic influences³⁻⁶, gene expression abnormalities^{7,8}, and neuronal
 61 dysfunctions^{9,10} associated with these conditions overlap, suggesting a common underlying
 62 biological basis. However, the shared neurobiological alterations among the three conditions remain
 63 largely unknown.

64

65 A number of postmortem studies have indicated that pH is lower in the brains of patients with
 66 schizophrenia and bipolar disorder^{8,11-19}. Lower brain pH has also been observed in patients with
 67 ASD²⁰. In general, pH balance is considered critical for maintaining optimal health, and low pH has
 68 been associated with a number of somatic disorders²¹⁻²³. Therefore, it is reasonable to assume that
 69 lower pH may exert a negative impact on brain function and play a key role in the pathogenesis of
 70 various psychiatric disorders. However, lower brain pH has largely been considered as an
 71 artifact^{11,24-27} rather than a pathophysiology of such disorders^{13,28} for two main reasons. One is that
 72 chronic treatment with antipsychotics may affect brain pH by increasing lactate levels in rats¹¹, and
 73 most patients with these disorders receive chronic antipsychotics treatment throughout their lives.
 74 Another is that the agonistic state experienced before death decreases brain pH²⁵⁻²⁷ and this state
 75 could be different in patients with psychiatric disorders in comparison to controls. In human
 76 postmortem studies, it is technically difficult to exclude such confounding factors and to determine
 77 whether lower pH and increased lactate levels are indeed artifacts.

78

79 In the present study, we first confirmed that patients with schizophrenia and bipolar disorder exhibit
80 lower postmortem brain pH by conducting a meta-analysis of publicly available datasets. We then
81 measured brain pH in multiple mouse models of psychiatric disorders, which are devoid of such
82 confounding factors, in order to test the hypothesis that lower brain pH is a pathophysiology or an
83 endophenotype rather than an artifact in a subgroup of psychiatric disorders. We also measured
84 lactate levels, increases in which have frequently been linked to lower pH in the brains of patients
85 with psychiatric disorders^{11,13,29}. To our knowledge, the present study is the first to systematically
86 evaluate pH and lactate levels in mouse models of psychiatric disorders which eliminate the
87 confounds inherent in the human studies.

88

89 For the mouse models of psychiatric disorders, we focused on the ones reported to have
90 neurodevelopmental abnormalities in the brain, a part of which stay at pseudo-immature status³⁰⁻³³.
91 Specifically, we measured pH, lactate, and related metabolite levels in the postmortem brains of the
92 following mouse models: *schurri-2* (*Shn2*) knockout (KO) mice³⁴, forebrain-specific *calcineurin*
93 (*Cn*) KO mice³⁵⁻³⁸ and *neurogranin* (*Nrgn*) KO mice³⁹⁻⁴¹ as a model of schizophrenia; mice with
94 heterozygous knockout of the calcium/calmodulin-dependent protein kinase II alpha (*Camk2a* HKO
95 mice)^{42,43} as a model of bipolar disorder; and mice with heterozygous knockout of the long isoform
96 of chromodomain helicase DNA-binding protein 8 (*Chd8* HKO mice)³³ as a model of ASD.
97 These mouse strains have mutations in the genes implicated in the respective disorders and exhibit
98 molecular and behavioral abnormalities relevant to each condition, indicating good construct and

face validities, respectively (as described in detail in Materials and Methods).

Materials and Methods

Human data

Nine publicly available datasets were utilized in the present study (Supplementary Table 1): four schizophrenia datasets (GSE17612, GSE21935, GSE21138; NSWBTRC-SC⁴⁴), one bipolar disorder dataset (GSE5392), and three combined schizophrenia and bipolar disorder datasets (Stanley Medical Research Institute [SMRI] Collection A, SMRI Collection C, GSE35977, GSE53987). We obtained data regarding postmortem interval and age from these studies and data regarding medication from SMRI Collection A and SMRI Collection C.

Animals

We measured pH, lactate, and related metabolite levels in *Shn2* KO mice³⁴ (n = 5, 6 [controls, mutants]), *Cn* KO mice^{35–38} (n = 6, 5), *Nrgn* KO mice^{39–41} (n = 6, 5), *Camk2a* HKO mice^{42,43} (n = 5, 5) and *Chd8* HKO mice³³ (n = 5, 5), and their corresponding control mice. Both male and female mice were used in the present study, as no difference in pH between genders has been observed⁴⁵. All mice were between 19 and 45 weeks of age, and no significant difference in age was observed between controls and mutants within each strain. All animal experiments were approved by the Institutional Animal Care and Use Committee of Fujita Health University, based on the Law for the Humane Treatment and Management of Animals and the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain. Every effort was made to minimize the

120 number of animals used.

121

122 Shn2 was originally identified as a nuclear factor- κ B (NF- κ B) site-binding protein that tightly binds
 123 to the enhancers of major histocompatibility complex (MHC) class I genes and acts as an
 124 endogenous inhibitor of NF- κ B.⁴⁶ Its deficiencies in Shn2 may cause mild chronic inflammation in
 125 the brain and confer molecular, neuronal, and behavioral phenotypes relevant to schizophrenia in
 126 mice³⁴. Genome-wide association studies (GWASs) have identified a number of single nucleotide
 127 polymorphisms (SNPs) in the MHC region associated with schizophrenia^{47–49}. *Shn2* KO mice
 128 exhibit multiple abnormal behaviors related to schizophrenia, including increased locomotor
 129 activity, deficits in working memory, abnormal social behavior and impaired prepulse inhibition,
 130 which are commonly observed in *Cn* KO mice^{35,36} and *Nrgn* KO mice^{39–41} as well.

131

132 Calcineurin (Cn) is a calcium-dependent protein phosphatase and has been implicated in synaptic
 133 plasticity⁵⁰. *CN* has been reported to be associated with schizophrenia^{51–53}, and altered expression of
 134 calcineurin has been observed in the postmortem brains of patients with schizophrenia^{54,55}.
 135 Forebrain-specific *Cn* KO mice exhibit behavioral and cognitive abnormalities related to
 136 schizophrenia^{35,36}. Deficits in synaptic transmission in the frontal cortex have been suggested to be
 137 the underlying mechanism of working memory impairment in these mice³⁸. In addition, *Cn* KO
 138 mice exhibit disruption in ripple-associated information processing in the hippocampal CA1, which
 139 is implicated in cognitive impairments associated with schizophrenia³⁷.

140

141 Neurogranin (Nrgn) is a calmodulin-binding protein that modulates activity of the Camk2 protein
 142 downstream of *N*-methyl-d-aspartic acid (NMDA) receptors, and is implicated in synaptic
 143 plasticity³⁹. GWAS revealed significant association with SNPs located upstream of the *NRGN*⁴⁹, a
 144 finding recently confirmed by a large-scale GWAS⁵⁶, strongly suggesting that *NRGN* is a
 145 susceptibility gene for schizophrenia. *Nrgn* KO mice exhibit behavioral phenotypes related to
 146 schizophrenia³⁹⁻⁴¹.

147

148 Camk2 is a major downstream molecule of the NMDA receptor and is thought to play an essential
 149 role in synaptic plasticity. A recent study demonstrated genetic association of *CAMK2A* with
 150 bipolar disorder⁵⁷, and decreased mRNA expression has been observed in the frontal cortex of
 151 patients with bipolar disorder⁵⁸. In addition, the *Camk2a* gene was identified as one of the top
 152 candidate genes for bipolar disorder by a meta-analysis that integrated genetic and genomic data
 153 from both human and animal studies⁵⁹. At cellular level, neuronal hyperexcitability, which we
 154 previously detected in the hippocampal granule cells of *Camk2a* HKO mice⁴², was also found in the
 155 granule cell-like neurons differentiated from induced pluripotent stem cells (iPSCs) derived from
 156 patients with bipolar disorder⁶⁰. *Camk2a* HKO mice exhibit abnormal behaviors, such as deficits in
 157 social activity and working memory, which are analogous to those in patients with bipolar
 158 disorder/schizophrenia⁴². In addition, these mutant mice exhibit infradian cyclic activity levels,
 159 which may reflect infradian oscillation of mood substantially observed in patients with bipolar
 160 disorder⁴³. These findings suggest that *Camk2a* HKO mice have construct and face validity as a
 161 model of psychiatric disorders, especially of bipolar disorder.

162

163 Chd8, a member of the chromodomain helicase DNA-binding family of proteins, is known to act as
164 a chromatin-remodeling factor. Recent exome sequencing analyses have identified a number of *de*
165 *novo* mutations in a variety of genes in individuals with ASD, further revealing that *CHD8* is the
166 most frequently affected gene^{61–64}. *Chd8* HKO mice exhibit behavioral abnormalities reminiscent of
167 ASD in humans, including increased anxiety, increased persistence and abnormal social
168 interaction³³. *Chd8* deficiency induces aberrant activation of RE1 silencing transcription factor
169 (REST), a molecular brake of neuronal development, resulting in neurodevelopment abnormalities
170 in mice³³.

171

172 Collectively, these findings indicate that the mouse models used in the present study exhibit good
173 construct and face validities for their respective disorders.

174

175 **Measurement of pH**

176 Mice were sacrificed by cervical dislocation followed by decapitation, following which whole
177 brains were removed. The brains were immediately frozen in liquid nitrogen and stored at –80°C
178 until use. We measured brain pH basically as previously described¹¹. Briefly, the brains were
179 homogenized using the tissue homogenizer attached with a conical pestle in ice-cold distilled H₂O
180 (5 mL per 500 mg of tissue). The pH was measured using a pH meter (LAQUA F-72, Horiba
181 Scientific, Kyoto, Japan) after a three-point calibration at pH 4.0, pH 7.0 and pH 9.0. The pH of the
182 samples from control and mutant mice were read in triplicate for each sample. After pH

measurement, homogenates were immediately frozen and stored at -80°C until required for further analyses.

Lactate and glucose measurements

The concentration of lactate in the brain homogenates was determined using a multi-assay analyzer (GM7 MicroStat; Analox Instruments, London, UK) according to manufacturer's instructions. In our prior tests using several samples, we loaded 5 μl , 10 μl and 20 μl of supernatants to the instrument, observing that the measurements increased linearly in a volume-dependent manner ($r^2 > 0.99$). Based on these results, we used 20 μl of supernatants for each sample for lactate measurements. Likewise, glucose concentrations in 20 μl supernatant samples were determined using a multi-assay analyzer following calibration with 10 mmol/ml glucose standard solution. To normalize the effects of differences among strains, such as genetic background and age, z-scores for pH and lactate levels were calculated within each strain and used for the correlation analysis.

Pyruvate measurement

Pyruvate concentrations in 20 μl supernatant samples were determined using a pyruvate assay kit (BioVision, Mountain View, CA, USA). The fluorescence intensities were measured using a microplate reader equipped with a spectrofluorometer (ARVO X, PerkinElmer).

Adenosine diphosphate/adenosine triphosphate (ADP/ATP) ratio

An ADP/ATP Ratio Assay Kit (BioVision) was used to measure the ADP and ATP concentrations

204 according to the manufacturer's instructions.

205

206 **Bioinformatics analysis of transcriptome data**

207 We used the following mouse brain transcriptome data: frontal cortex and hippocampal dentate
 208 gyrus of *Shn2* KO mice (microarray)³⁴, hippocampal dentate gyrus of *Camk2a* HKO mice
 209 (microarray)⁶⁵, and whole brains of *Chd8* HKO mice (RNA-sequencing)³³. Gene expression
 210 patterns of the frontal cortex of *Camk2a* HKO mice (n = 6, 6) and hippocampal DG of *Cn* KO mice
 211 (n = 6, 6) were analyzed via microarray (Mouse Genome 430 2.0 Array; Affymetrix, Santa Clara,
 212 CA, USA), as previously described³⁴. Gene expression patterns of the frontal cortex and
 213 hippocampal DG of *Nrgn* KO mice (n = 5, 5) were analyzed via RNA-sequencing using the HiSeq
 214 platform basically according to the manufacturer's instructions (Illumina, San Diego, CA, USA).
 215 Genes with an absolute fold change > 1.2 and a *t*-test *P*-value < 0.05 (mutants vs. controls; without
 216 correction for multiple testing) were imported into the bioinformatics tool BaseSpace (Illumina),
 217 with which the gene expression data obtained from different platforms can be matched. Genes with
 218 altered expression in at least four out of the eight datasets (yielding 80 features; Supplementary
 219 Table 2) were selected based on the criteria of the BaseSpace tool and assessed for enrichment in
 220 biological themes using the DAVID functional annotation clustering tool, ADGO, and GOToolBox,
 221 in which the default feature listings and algorithm settings were used.

222

223 **Results**

224 **Meta-analysis of human brain pH studies**

225 We first re-evaluated the results of postmortem studies of brain pH in patients with schizophrenia
 226 and bipolar disorder by conducting a meta-analysis of publicly available datasets. We searched the
 227 National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO),
 228 ArrayExpress, and Stanley Medical Research Institute (SMRI) databases, and found nine studies
 229 that included individual brain pH data from patients with schizophrenia, bipolar disorder, or both as
 230 well as from healthy control participants (Supplementary Table 1). A two-way analysis of variance
 231 (ANOVA) revealed a significant effect of condition ($F_{2,645} = 3.35$, $P = 3.09 \times 10^{-10}$) and study ($F_{8,645}$
 232 $= 10.00$, $P = 2.00 \times 10^{-16}$) as well as between the two factors ($F_{11,645} = 47.66$, $P = 0.043$) (Figure 1).
 233 *Post hoc* comparisons with Tukey's honest significant difference test indicated a lower brain pH in
 234 both patients with schizophrenia ($P < 1.0 \times 10^{-7}$) and bipolar disorder ($P = 0.00036$) compared to
 235 healthy controls, and no significant difference between the two conditions ($P = 0.56$). The results of
 236 our meta-analysis therefore support the finding of lower brain pH in patients with schizophrenia and
 237 bipolar disorder.

238
 239 Brain pH was not correlated with lifetime use of antipsychotics (measured as fluphenazine
 240 equivalents) in a schizophrenia group (Pearson's $r = -0.27$, $p = 0.12$), a bipolar disorder group ($r =$
 241 -0.27 , $P = 0.13$), or a group including both conditions ($r = -0.15$, $P = 0.23$) from the SMRI
 242 Collection A cohort (Supplementary Figure 1a). No correlation between pH and antipsychotics was
 243 replicated in a separate cohort from SMRI Collection C (schizophrenia: $r = 0.16$, $P = 0.58$; bipolar
 244 disorder: $r = 0.036$, $P = 0.90$; both: $r = 0.090$, $P = 0.64$) (Supplementary Figure 1b). These suggest
 245 that antipsychotics treatment may not affect pH in the postmortem brains of patients with

schizophrenia and bipolar disorder. On the other hand, in the combined data, pH was positively correlated with postmortem interval ($r = 0.13$, $P = 0.0010$; Supplementary Figure 1c) and negatively correlated with age ($r = -0.13$, $P = 0.00092$; Supplementary Figure 1d), suggesting that these factors may contribute to the changes in the pH of postmortem brains.

Lower pH and increased lactate levels in the postmortem brain of mouse models of schizophrenia, bipolar disorder, and ASD

The confounding factors identified in previous studies^{11,26} are beyond investigator's control in human postmortem brain studies. We therefore measured pH and lactate levels in the brains of mouse models of schizophrenia (*Shn2* KO, *Cn* KO, *Nrgn* KO mice), bipolar disorder (*Camk2a* HKO mice), and ASD (*Chd8* HKO mice). All the mice used were drug-naïve and sacrificed by cervical dislocation (controlling for agonal state differences). The removed brains were snap-frozen within a few minutes (controlling for postmortem interval differences). Brain pH was significantly lower in all five mutant strains examined relative to the corresponding controls (*Shn2* KO, 7.17 ± 0.0060 , controls [Con], 7.20 ± 0.056 , $P = 0.0083$; *Cn* KO, 7.08 ± 0.0057 , Con, 7.13 ± 0.0080 , $P = 0.0014$; *Nrgn* KO, 7.10 ± 0.017 , Con, 7.16 ± 0.0080 , $P = 0.0090$; *Camk2a* HKO, 7.14 ± 0.0093 , Con, 7.21 ± 0.0090 , $P = 0.0014$; *Chd8* HKO, 7.08 ± 0.0066 , Con, 7.12 ± 0.0031 , $P = 0.00080$) (Figure 2a).

Significantly higher levels of lactate were observed in the postmortem brains of all mutant mice strains compared to corresponding controls (*Shn2* KO, 2.98 ± 0.080 mM, Con, 2.55 ± 0.076 mM, P

267 = 0.0038; *Cn* KO, 3.24 ± 0.051 mM, Con, 2.90 ± 0.073 mM, $P = 0.0052$; *Nrgn* KO, 2.98 ± 0.11
 268 mM, Con, 2.58 ± 0.054 mM, $P = 0.0080$; *Camk2a* HKO, 2.86 ± 0.024 mM, Con, 2.58 ± 0.037 mM,
 269 $P = 0.00024$; *Chd8* HKO, 3.04 ± 0.081 mM, Con, 2.58 ± 0.086 mM, $P = 0.0046$; Figure 2b).
 270 Analysis of the combined data expressed as the *z*-score revealed that pH was significantly
 271 negatively correlated with lactate levels (Pearson's $r = -0.65$, $P = 1.19 \times 10^{-7}$; Figure 2c).

272
 273 Lactate is formed from pyruvate during glycolysis. We therefore measured pyruvate levels in
 274 mutant mouse brains and observed that levels were significantly increased in *Shn2* KO ($P = 0.011$),
 275 *Cn* KO ($P = 0.046$), *Nrgn* KO ($P = 0.011$) and *Chd8* HKO mice ($P = 0.0036$) and showed increased
 276 tendency in *Camk2a* HKO mice ($P = 0.068$) (Figure 2d). Glucose levels remained unchanged in
 277 mutant mice relative to controls (Figure 2e), suggesting glucose supply/demand ratio in the brain
 278 may be comparable in these mouse models. The ADP/ATP ratio was decreased in *Nrgn* KO mice (P
 279 = 0.035) and increased in *Chd8* HKO mice ($P = 0.047$) (Figure 2f), suggesting a contrasting energy
 280 consumption ratio in mouse models of schizophrenia and ASD.

281
 282 We then analyzed transcriptome data (Supplementary Table 2) in order to investigate the potential
 283 underlying molecular mechanisms of increased lactate levels in mutant mouse brains. The
 284 transcriptome data from five mouse strains revealed an enrichment in Wnt- and epidermal growth
 285 factor (EGF)-related pathways when analyzed with DAVID software (Supplementary Table 3).
 286 Enrichment in Wnt-related pathways was replicated in the analyses using other bioinformatics tools
 287 (ADGO and GOToolBox) using different statistical methods (Supplementary Table 3).

288

289 Since lactate is produced via glycolytic pathways in astrocytes in the brain⁶⁶, we analyzed the
290 transcriptome data of mutant mice with particular focus on glycolysis-related genes (Gene Ontology
291 Consortium database), as well as those related to pyruvate metabolism. The results of the targeted
292 gene expression analyses suggest that elevated glycolysis and pyruvate metabolism shifting toward
293 lactate synthesis occurs in the brains of mutant mice, especially in *Shn2* KO and *Camk2a* HKO
294 mice (Supplementary Table 4; Supplementary Figure 2).

295

296 Discussion

297 In the present study, we confirmed lower pH in the postmortem brains of patients with
298 schizophrenia and bipolar disorder by conducting a meta-analysis of existing datasets. Lower pH
299 was also observed in five different mouse models of psychiatric disorders, all of which were
300 drug-naïve and were controlled for other confounding factors, such as agonal state and postmortem
301 interval. We also observed increased lactate levels in the brains of mutant mice, as well as a highly
302 significant negative correlation between pH and lactate levels, which is consistent with the findings
303 of previous human postmortem studies¹¹. These results suggest that lower pH and increased lactate
304 levels represent components of the underlying pathophysiology of the diseases rather than mere
305 artifacts.

306

307 Researches have revealed that brain acidosis influences a number of brain functions, such as anxiety,
308 mood, and cognition⁶⁷. Acidosis may affect the structure and function of several types of brain cells,

including the electrophysiological functioning of GABAergic neurons⁶⁸ and morphological properties of oligodendrocytes⁶⁹. Alterations in these types of cells have been well-documented in the brains of patients with schizophrenia, bipolar disorder, and ASD^{70,71} and may underlie some of the cognitive deficits associated with these disorders. Deficits in GABAergic neurons and oligodendrocytes have been identified in the mouse models of the disorders, including *Shn2* KO mice^{30,34}. Brain acidosis may therefore be associated with deficits in such cell types in schizophrenia, bipolar disorder, and ASD.

A previous study indicated that chronic treatment with antipsychotics increases lactate levels in the rat cerebral cortex¹¹, suggesting that such increases may be medication-related. The authors of the report, however, found no significant correlation between lactate levels and history of antipsychotic use (which was represented by chlorpromazine equivalents) in the postmortem brains of patients with schizophrenia¹¹. In addition, increased lactate levels have been observed in the anterior cingulate of medication-free patients with bipolar disorder in *in vivo* spectroscopic imaging studies⁷². Furthermore, studies utilizing animal models of psychiatric disorders—including the current study—have identified increased lactate levels in mutant mouse brains⁷³. In addition, increased lactate levels were associated with lower pH in the brains of mutant mice, consistent with findings from previous studies on patients with schizophrenia^{11,13}. Lower brain pH has also been observed in the medication-free patients with bipolar disorder²⁸. Although it remains possible that antipsychotic treatment increases lactate levels and lowers pH in the brain, the aforementioned findings suggest that such changes may occur as primary features of schizophrenia and bipolar

330 disorder.

331

332 Interestingly, we observed that Wnt- and EGF-related pathways, which are highly implicated in
 333 somatic and brain cancers⁷⁴, are enriched in the genes whose expressions were altered among the
 334 five mutant mouse strains. It is known that cancer cells display high rates of glycolysis, resulting in
 335 high lactate and pyruvate levels, even in normoxia⁷⁵; this phenomenon has been referred to as the
 336 Warburg effect. Genes whose expression is known to positively regulate the Warburg effect, such
 337 as *Hk2*⁷⁶, *Hif1a*⁷⁵, and *Pfkfb3*,⁷⁷ were increased in the brains of any of mouse models examined in
 338 the present study, while expression of *Prkaa1*, a negative regulator of the Warburg effect⁷⁸, was
 339 decreased (Supplementary Table 2). These findings raise the possibility that elevated glycolysis
 340 underlies the increased lactate and pyruvate levels in the brains of the mouse models of
 341 schizophrenia, bipolar disorder, and ASD. The results of the targeted gene expression analyses
 342 conducted in the present study also support the hypothesis. Glycolysis is also stimulated by the
 343 uptake of glutamate in astrocytes following neuronal excitation⁷⁹. Dysregulation of the
 344 excitation-inhibition balance has been proposed as a candidate cause of schizophrenia, bipolar
 345 disorder, and ASD^{80,81}. A shift in the balance towards excitation would result in increased energy
 346 expenditure and may lead to increased glycolysis. Indeed, *Shn2* KO mice exhibit higher glutamate
 347 levels in the hippocampus³⁴. *In vivo* metabolite measurements have indicated that increased
 348 glycolysis occurs in the brains of patients with bipolar disorder^{29,72}, while gene ontology analysis of
 349 microarray data has indicated that decreased glycolysis occurs in the brains of patients with
 350 schizophrenia¹³. Further studies are required to determine whether altered glycolysis rate is

associated with increased lactate levels.

It has been indicated that lactate levels in the mouse brain rapidly increase after at least 1 min of decapitation as compared to *in vivo* fixation by focused microwave irradiation, which is regarded as a consequence of enhanced glycolysis under oxygen-deprived conditions⁸². While the current findings may differ from those obtained under physiological conditions, they may reflect functional changes, such as the activation of astrocytes^{34,40}, which represent the main source of lactate production in the brain.

Brain pH is associated with notable changes in gene expression^{16,26,45,83} and has hence been considered as a confound for investigating changes in gene expression related to the pathophysiology of psychiatric disorders. Therefore, substantial effort has been made to match the tissue pH between patients and controls. Given that lower brain pH is a pathophysiology of certain conditions, pH-dependent changes in gene expression would not be negligible when attempting to elucidate the molecular basis of the conditions. It has been known that gene expression patterns are partially similar across diseases such as schizophrenia, bipolar disorder, and ASD^{7,8}. Lower pH may underlie the similarities of gene expression patterns. Thus, pH may be an important factor in the elucidation of molecular alternations in the brains of patients with these psychiatric conditions.

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Author contributions

Conceived and designed the experiments: HH and TM. Contributed materials: VC, YK, TT, FH, KH, SI, IG, GC, KN and CW. Analyzed the data: HH and TM.

Conflict of interest

Dr. Tsuyoshi Miyakawa and Dr. Cynthia Shannon Weickert both receive research grants from Astellas Pharma Inc. Dr. Cynthia Shannon Weickert is a consultant for Lundbeck, Australia Pty Ltd. Other authors have no conflict of interests to declare.

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Figure legends

Figure 1. Lower pH in the postmortem brains of patients with schizophrenia and bipolar disorder revealed by meta-analysis of publicly available data

Box plot of pH in the brain of control participants (*white box*), patients with schizophrenia (*red box*) and patients with bipolar disorder (*blue box*). # $P = 0.017$; One-way analysis of variance (ANOVA)/Tukey's honest significant difference test. *¹ $P = 8.0 \times 10^{-6}$, *² $P = 1.3 \times 10^{-4}$, *² $P = 0.027$; Student's *t*-test. The boxes represent the interquartile range between first and third quartiles, the whiskers the maximum and minimum values and the circles population outliers.

Figure 2. Negative correlation between lower pH and increased lactate levels in the postmortem brains of mouse models of psychiatric disorders

Bar graphs of pH (**a**), lactate levels (**b**), pyruvate levels (**d**), glucose levels (**e**), and ADT/ATP ratio (**f**) in the brains of *Shn2* KO, *Cn* KO, *Nrgn* KO, *Camk2a* HKO, and *Chd8* HKO mice and their corresponding controls (average $\pm$ SEM). Each plot represents individual mouse values. (**c**) Scatter plot showing correlations between pH and lactate levels in the mouse brain. * $P < 0.05$, ** $P < 0.01$; Student's *t*-test. SEM: standard error of the mean. ADP: adenosine diphosphate; ATP: adenosine triphosphate.

Supplementary information

Supplementary Figure 1. Correlations between pH and lifetime antipsychotic use, postmortem interval, and age

Scatter plots showing correlations between pH and lifetime antipsychotic (fluphenazine equivalents) use in the SMRI collection A **(a)** and SMRI collection C **(b)** datasets. Scatter plots showing the correlation between pH and postmortem interval (controls: $r = 0.027$, $P = 0.66$; schizophrenia: $r = 0.27$, $P = 2.1 \times 10^{-5}$; bipolar disorder: $r = 0.14$, $P = 0.085$; Total: $r = 0.13$, $P = 0.0010$) **(c)**, and age (controls: $r = -0.14$, $P = 0.021$; schizophrenia: $r = -0.22$, $P = 0.00075$; bipolar disorder: $r = 0.14$, $P = 0.096$; Total: $r = -0.13$, $P = 0.00092$) **(d)**. SMRI: Stanley Medical Research Institute.

Supplementary Figure 2. Potentially elevated glycolysis in the brains of mouse models of psychiatric disorders

Glycolysis-related genes whose expression was altered in the brains of mouse models of psychiatric disorders were mapped in a schematic of the glycolysis pathway.

Supplementary Table 1. Patient characteristics

Antipsychotic dose (mg) is measured as fluphenazine equivalents. M, male; F, female; na, not available.

Supplementary Table 2. Genes whose expression was altered in the brains of mouse models of psychiatric disorders

657 Genes whose expression was altered in at least four out of eight mouse datasets were processed for
658 pathway analyses.

659

660 **Supplementary Table 3. Pathway analyses of the genes whose expression was altered in the**
661 **brains of mouse models of the psychiatric disorders using DAVID, ADGO, and GoToolBox**

662 The top 20 pathways (ranked based on the *P*-value) are shown for each analysis.

663

664 **Supplementary Table 4. Expression patterns of genes encoding enzymes related to glycolysis**
665 **pathway in the brains of mouse models of psychiatric disorders**

Figure 1

Figure 2