Neuropsychopharmacology (2018) 43, 459–468
Official journal of the American College of Neuropsychopharmacology

www.neuropsychopharmacology.org

Decreased Brain pH as a Shared Endophenotype of Psychiatric Disorders

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Although the brains of patients with schizophrenia and bipolar disorder exhibit decreased brain pH relative to those of healthy controls upon postmortem examination, it remains controversial whether this finding reflects a primary feature of the diseases or is a result of confounding factors such as medication and agonal state. To date, systematic investigation of brain pH has not been undertaken using animal models that can be studied without confounds inherent in human studies. In the present study, we first reevaluated the pH of the postmortem brains of patients with schizophrenia and bipolar disorder by conducting a meta-analysis of existing data sets from 10 studies. We then measured pH, lactate levels, and related metabolite levels in brain homogenates from five neurodevelopmental mouse models of psychiatric disorders, including schizophrenia, bipolar disorder, and autism spectrum disorder. All mice were drug naive with the same agonal state, postmortem interval, and age within each strain. Our meta-analysis revealed that brain pH was significantly lower in patients with schizophrenia and bipolar disorder than in control participants, even when a few potential confounding factors (postmortem interval, age, and history of antipsychotic use) were considered. In animal experiments, we observed significantly lower pH and higher lactate levels in the brains of model mice relative to controls, as well as a significant negative correlation between pH and lactate levels. Our findings suggest that lower pH associated with increased lactate levels is not a mere artifact, but rather implicated in the underlying pathophysiology of schizophrenia and bipolar disorder.

Neuropsychopharmacology (2018) **43,** 459–468; doi:10.1038/npp.2017.167; published online 6 September 2017

INTRODUCTION

Schizophrenia, bipolar disorder, and autism spectrum disorder (ASD) are highly heritable psychiatric conditions, with clinical features transcending diagnostic categories (Hyman, 2010; Insel *et al*, 2010). Accumulating evidence indicates that some genetic influences (Carroll and Owen, 2009; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013a, b; Lotan *et al*, 2014), gene expression abnormalities (Ellis *et al*, 2016; Shao and Vawter, 2008), and neuronal dysfunctions (Goodkind *et al*, 2015; Yahata *et al*, 2016) associated with these conditions overlap, suggesting a common underlying biological basis. However, the shared neurobiological alterations among the three conditions remain largely unknown.

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Received 4 May 2017; revised 14 July 2017; accepted 1 August 2017; accepted article preview online 4 August 2017

A number of postmortem studies have indicated that pH is decreased in the brains of patients with schizophrenia and bipolar disorder (Guillozet-Bongaarts *et al*, 2014; Halim *et al*, 2008; Iwamoto *et al*, 2005; Lipska *et al*, 2006; Mistry *et al*, 2013; Prabakaran *et al*, 2004; Ryan *et al*, 2006; Shao and Vawter, 2008; Sun *et al*, 2006; Torrey *et al*, 2005). Decreased brain pH has also been observed in patients with ASD (Young *et al*, 2011). In general, pH balance is considered critical for maintaining optimal health, and low pH has been associated with a number of somatic disorders (Kraut and Madias, 2014; Narins and Emmett, 1980; Posner and Plum, 1967). Therefore, it is reasonable to assume that lower pH may exert a negative impact on brain function and play a key role in the pathogenesis of various psychiatric disorders. However, lower brain pH has largely been considered as an artifact rather than a pathophysiology of such disorders. Animal studies have indicated that chronic treatment with antipsychotics may affect brain pH by increasing lactate levels (Halim *et al*, 2008), and most patients with these disorders receive chronic antipsychotic treatment throughout their lives. In addition, the agonal state experienced before death decreases brain pH (Li *et al*, 2004; Tomita *et al*, 2004; Vawter *et al*, 2006), and this state may differ between patients with psychiatric disorders and controls. In human postmortem studies, it is technically difficult to exclude such confounding factors and to determine whether decreased pH and increased lactate levels are indeed artifacts.

In the present study, we first confirmed that patients with schizophrenia and bipolar disorder exhibit lower postmortem brain pH by conducting a meta-analysis of publicly available data sets. We then measured brain pH in multiple mouse models of psychiatric disorders, which are devoid of such confounding factors, in order to test the hypothesis that decreased brain pH is a pathophysiological manifestation/endophenotype of these disorders rather than a mere artifact. We also measured lactate levels, increases in which have frequently been linked to decreased pH in the brains of patients with psychiatric disorders (Halim *et al*, 2008; Prabakaran *et al*, 2004; Stork and Renshaw, 2005). To our knowledge, the present study is the first to systematically evaluate pH and lactate levels in mouse models of psychiatric disorders that eliminate the confounds inherent in human studies.

We focused on mouse models of psychiatric disorders reported to exhibit neurodevelopmental abnormalities in the brain, a part of which stay at a pseudo-immature state (Hagihara *et al*, 2013). Specifically, we measured pH, lactate, and related metabolite levels in the postmortem brains of the following mouse models: *schnurri-2* (*Shn2*) knockout (KO) mice (Takao *et al*, 2013), forebrain-specific *calcineurin* (*Cn*) KO mice (Cottrell *et al*, 2013; Miyakawa *et al*, 2003; Suh *et al*, 2013; Zeng *et al*, 2001), and *neurogranin* (*Nrgn*) KO mice (Huang *et al*, 2006; Huang and Huang, 2012; Pak *et al*, 2000) as a model of schizophrenia; mice with heterozygous knockout of the calcium/calmodulin-dependent protein kinase II *a* (*Camk2a* HKO mice) (Hagihara *et al*, 2016; Yamasaki *et al*, 2008) as a model of bipolar disorder; and mice with heterozygous knockout of the long isoform of chromodomain helicase DNA-binding protein 8 (*Chd8* HKO mice) (Katayama *et al*, 2016) as a model of ASD. These mouse strains are characterized by mutations in genes implicated in the respective disorders and exhibit molecular and behavioral abnormalities relevant to each condition, indicating good construct and face validities, respectively (as described in detail in the Supplementary Materials and Methods).

MATERIALS AND METHODS

Human Data

We obtained pH data of healthy individuals and patients with schizophrenia and bipolar disorder from the Stanley Medical Research Institute (SMRI) database (https://www.stanleygenomics.org). Duplicate data among studies in the database were eliminated. In addition, we comprehensively searched the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database for studies reporting individual pH data using the following terms: schizophrenia, bipolar disorder, autism. We also used pH data for healthy individuals and patients with schizophrenia obtained from the New South Wales Brain Tissue Resource Centre schizophrenia cohort (NSWBTRC-SC) (Fillman *et al*, 2013), and from a study by Dean *et al* (2016). Altogether, 10 publicly available data sets were utilized in the present study (Supplementary Table S1): five schizophrenia data sets (GSE17612, GSE21935, GSE21138, NSWBTRC-SC (Fillman *et al*, 2013), and Dean *et al*, 2016)), one bipolar disorder data set (GSE5392), and four combined schizophrenia and bipolar disorder data sets (SMRI Collection A, SMRI Collection C, GSE35977, GSE53987). In addition, we used data regarding postmortem interval and age from these data sets, as well as data regarding medication from SMRI Collection A, SMRI Collection C, Dean *et al*, 2016, and GSE5392.

Animals

We measured pH, lactate, and related metabolite levels in *Shn2* KO mice (Takao *et al*, 2013) ($n = 5$, 6 (controls, mutants)), *Cn* KO mice (Cottrell *et al*, 2013; Miyakawa *et al*, 2003; Suh *et al*, 2013; Zeng *et al*, 2001) ($n = 6$, 5), *Nrgn* KO mice (Huang *et al*, 2006; Huang and Huang, 2012; Pak *et al*, 2000) ($n = 6$, 5), *Camk2a* HKO mice (Hagihara *et al*, 2016; Yamasaki *et al*, 2008) ($n = 5$, 5), *Chd8* HKO mice (Katayama *et al*, 2016) ($n = 5$, 5), *disrupted-in-schizophrenia 1* (*Disc1*)-*L100P* mutant mice ($n = 6$, 6), *Disc1-Q31L* mutant mice ($n = 6$, 6)(Shoji *et al*, 2012), voltage-gated calcium channel β-anchoring and -regulatory protein (*Barp*) KO mice (Nakao *et al*, 2015) ($n = 10$, 10), and their corresponding control littermates. *Shn2* KO and wild-type control mice were obtained by breeding heterozygotes with a C57BL/6J background and those with a BALB/cA background (Takao *et al*, 2013). All other strains were characterized by a C57BL/6J background. Both male and female mice were used in the present study, as no difference in pH has been observed between sexes (Catts *et al*, 2005). 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 (*Shn2* KO, 39.3 ± 3.0 weeks, controls (Con), 34.9 ± 1.2 weeks, $P = 0.19$; *Cn* KO, 20.6 ± 0.47 weeks, Con, 20.6 ± 0.36 weeks, $P = 0.92$; *Nrgn* KO, 32.9 ± 1.2 weeks, Con, 31.0 ± 0.14 weeks, $P = 0.12$; *Camk2a* HKO, 33.0 ± 0.052 weeks, Con, 36.2 ± 1.9 weeks, $P = 0.13$; *Chd8* HKO, 41.0 weeks, Con, 41.0 weeks; *Disc1-L100P* Mut, 29.0 ± 0.41 weeks, Con, 29.0 ± 0.40 weeks, $P = 0.97$; *Disc1-Q31L* Mut, 36.1 ± 0.60 weeks, Con, 36.3 ± 0.66 weeks, $P = 0.80$; *Barp* KO, 20.3 weeks, Con, 20.3 weeks). 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 number of animals used. *Shn2* KO, *Cn* KO, *Nrgn* KO, *Camk2a* HKO, and *Chd8* HKO mice—but not *Disc1-L100P* mutant and *Disc1-Q31L* mutant, or *Barp* KO mice—exhibit good construct and face validities for schizophrenia, bipolar disorder, and ASD. Further details are included in the Supplementary Materials and Methods (Supplementary Table S2).

Measurement of pH

Mice were killed via cervical dislocation and decapitation, following which whole brains were removed. The brains were immediately frozen in liquid nitrogen and stored at –80 °C until use. We measured brain pH as previously described (Catts *et al*, 2005; Halim *et al*, 2008). Briefly, mouse brains were homogenized using a tissue homogenizer equipped with a conical pestle in ice-cold distilled H_2O (5 ml per 500 mg of tissue). The pH was measured using a pH meter (LAQUA F-72, Horiba Scientific, Kyoto, Japan) after a three-point calibration at pH 4.0, pH 7.0, and pH 9.0. The pH experiments were performed in triplicate for each sample, following which 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) in accordance with the manufacturer's instructions. In our prior tests using several samples, we loaded 5, 10, and 20 μl of supernatant to the instrument, observing linear, volume-dependent increases in the measured values ($r^2 > 0.99$). Based on these results, we used 20 μl of supernatant for lactate measurements. Similarly, glucose concentrations in 20 μl supernatant samples were determined using a multiassay analyzer following calibration with 10 mmol/ml glucose standard solution. To normalize the effects of differences in genetic background and age among strains, *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). 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, in accordance with the manufacturer's instructions.

Data Analysis

Human data. We used all data obtained and conducted two-way analyses of variance (ANOVA) and covariance (ANCOVA) for pH, factoring in diagnosis and data set using SAS Studio software version 3.5 (SAS Institute, Cary, NC). Tukey's honest significant difference (HSD) *post hoc* test after ANOVA or ANCOVA was also employed to assess the significance of differences between the mean values of diagnostic groups.

Because of differences in methods used to calculate equivalent doses among the four data sets (fluphenazine *vs* chlorpromazine equivalent), we calculated *Z*-scores for lifetime antipsychotic use. *Z*-score transformation—a traditional method of data normalization for direct comparison between different samples and conditions—was applied for each antipsychotic equivalent value and pH value using individual participant data within each of four data sets, according to the following formula:

$$Z\text{-score} = (\text{value}_P - \text{ mean value}_{P1\ldots Pn})/\text{standard deviation}_{P1\ldots Pn},$$

where P is any pH and $P1\ldots P_n$ represent the aggregate measure of all antipsychotic equivalent or pH values.

Mouse data. Student's *t*-test was employed to assess the significance of differences between the mean values of controls and mutants in combination with the Bonferroni–Holm correction for repeated measurements. *Z*-scores for pH and lactate levels were calculated within strains as described above.

Transcriptome Analysis and Bioinformatics Analysis

We used the following mouse brain transcriptome data: frontal cortex and hippocampal dentate gyrus (DG) of *Shn2* KO mice (microarray) (Takao *et al*, 2013), hippocampal DG of *Camk2a* HKO mice (microarray) (Hagihara *et al*, 2009), and whole brains of *Chd8* HKO mice (RNA-sequencing) (Katayama *et al*, 2016). Gene expression patterns in the frontal cortex of *Camk2a* HKO mice ($n=6$, 6) and hippocampal DG of *Cn* KO mice ($n=6$, 6) were analyzed via microarray (Mouse Genome 430 2.0 Array; Affymetrix, Santa Clara, CA), as previously described (Takao *et al*, 2013). Gene expression patterns in the frontal cortex and hippocampal DG of *Nrgn* KO mice ($n=5$, 5) were analyzed via RNA-sequencing using the HiSeq platform in accordance with the manufacturer's instructions (Illumina, San Diego, CA). A total of eight transcriptome data sets were used in the present study. Genes with an absolute fold change >1.2 and a *t*-test *P*-value <0.05 (mutants *vs* controls; without correction for multiple testing) were imported into the bioinformatics tool BaseSpace (Illumina), with which the gene expression data obtained from different platforms were matched (Hagihara *et al*, 2014). Genes meeting the above criteria are included in Supplementary Table S3. Genes with altered expression in at least four of the eight data sets (yielding 80 features; Supplementary Table S3) were selected based on the criteria of the BaseSpace tool and assessed for enrichment in biological themes using the DAVID functional annotation clustering tool, ADGO, and GOToolBox, in which the default feature listings and algorithm settings were used.

RESULTS

Lower pH in the Postmortem Brains of Patients with Schizophrenia and Bipolar Disorder

We first performed a meta-analysis of postmortem studies regarding brain pH in patients with schizophrenia and bipolar disorder that consisted of nine schizophrenia data sets and five bipolar disorder data sets (Figure 1 and Supplementary Table S1). A two-way ANOVA revealed significant effects of diagnosis ($F_{2,\,694}=22.01$, $P=5.32\times10^{-10}$) and data set ($F_{9,\,694}=17.93$, $P<2.00\times10^{-16}$), although no interaction was observed between the two factors ($F_{12,\,694}=1.70$, $P=0.063$; Figure 1). The *post hoc* comparisons with Tukey's HSD test indicated that patients with schizophrenia (adjusted $P<1.0\times10^{-7}$) and bipolar disorder (adjusted $P=9.6\times10^{-6}$)

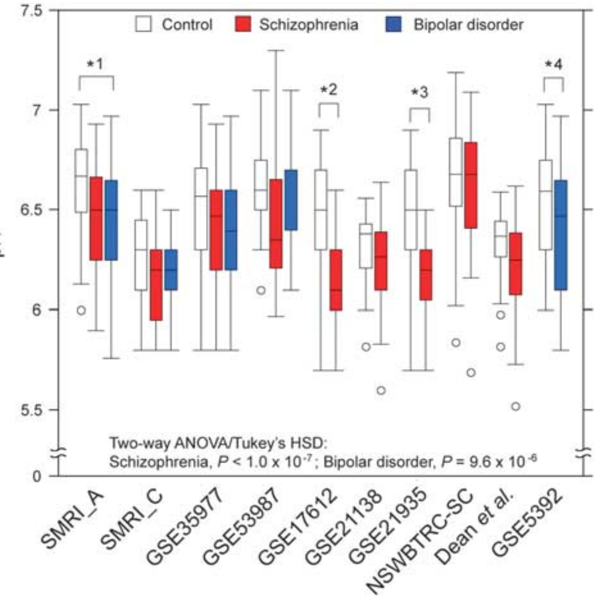

Figure 1 Lower pH in the postmortem brains of patients with schizophrenia and bipolar disorder. Box plot of brain pH in control participants (white box), patients with schizophrenia (red box), and patients with bipolar disorder (blue box). The boxes represent the interquartile range between first and third quartiles, whereas the whiskers represent the maximum and minimum values, and the circles represent population outliers. $^{*1}P=0.020$, $^{*2}P<0.0001$, $^{*3}P=0.0001$, $^{*4}P=0.027$; ANOVA/Tukey's *post hoc* test within each data set.

exhibited lower brain pH than healthy control. However, no significant difference in brain pH was observed between patients with schizophrenia and bipolar disorder (adjusted $P=0.88$). Similar results were obtained with *Z*-score-transformed data: pH was significantly lower in patients with schizophrenia (adjusted $P<1.0\times10^{-7}$) and bipolar disorder (adjusted $P=1.59\times10^{-5}$) than in healthy controls, and no significant difference was observed between patients with schizophrenia and bipolar disorder (adjusted $P=0.85$) (Supplementary Figure S1).

We observed no significant interactions between diagnosis and postmortem interval/age in either schizophrenia (postmortem interval: $F_{1, 485}=2.56$, $P=0.11$; age: $F_{1, 485}=0.93$, $P=0.34$) or bipolar disorder data sets (postmortem interval: $F_{1, 295}=0.63$, $P=0.43$; age: $F_{1, 295}=1.07$, $P=0.30$). Hence, we performed correlation analyses between pH and such variables using the combined data of patients and controls to test their effects on pH. In schizophrenia data sets, significant correlations were observed between pH values and age ($r=-0.17$, $P=0.00013$), but not between pH and postmortem interval ($r=0.064$, $P=0.14$). In bipolar disorder data sets, no significant correlations were observed between pH and postmortem interval ($r=0.072$, $P=0.20$), or between pH and age ($r=0.032$, $P=0.57$) (Supplementary Figure S2). Therefore, we performed an ANCOVA (factors: diagnosis and data set) on schizophrenia samples with age as a covariate that revealed a significant main effect of diagnosis ($F_{1, 485}=6.16$, $P=0.013$). However, no significant interaction effects were observed for these factors ($F_{8, 485}=0.35$, $P=0.95$). In bipolar disorder data sets, as no significant correlations were observed between pH and potential confounding factors, we performed an ANOVA to examine the effect of diagnosis on pH. Our analysis revealed a significant main effect of diagnosis ($F_{1, 305}=9.11$, $P=0.0028$), although no significant interaction effects were observed ($F_{4, 305}=0.68$, $P=0.60$). The *post hoc* analyses revealed that pH was lower in the brains of patients with schizophrenia ($P<0.0001$) and bipolar disorder ($P=0.0028$) than in those of healthy controls. Furthermore, *Z*-score-based meta-analysis of six data sets revealed no significant correlation between pH and lifetime use of antipsychotics ($r=-0.13$, $P=0.094$; Supplementary Figure S3), suggesting that antipsychotic treatment is not a major contributing factor affecting pH in the postmortem brains of patients with schizophrenia and bipolar disorder. Collectively, the results of our meta-analysis support the notion that lower brain pH is a pathological feature of schizophrenia and bipolar disorder rather than an artifact.

Lower pH and Increased Lactate Levels in the Postmortem Brain of Mouse Models of Schizophrenia, Bipolar Disorder, and ASD

The potential confounding factors identified in previous studies (Halim *et al*, 2008; Tomita *et al*, 2004) are beyond the investigator's control in postmortem studies of the human brain. 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 mice used were drug naive and killed via cervical dislocation, following which the removed brains were snap-frozen within a few minutes, allowing us to control for differences in agonal state and postmortem interval differences. Brain pH was significantly lower in all five mutant strains than in the corresponding controls (*Shn2* KO, 7.17 ± 0.0060, Con, 7.20 ± 0.0056, $P=0.017$; *Cn* KO, 7.08 ± 0.0057, Con, 7.13 ± 0.0080, $P=0.0055$; *Nrgn* KO, 7.10 ± 0.017, Con, 7.16 ± 0.0080, $P=0.017$; *Camk2a* HKO, 7.14 ± 0.0093, Con, 7.21 ± 0.0090, $P=0.0055$; *Chd8* HKO, 7.08 ± 0.0066, Con, 7.12 ± 0.0031, $P=0.0040$; Figure 2a).

Significantly higher levels of lactate were observed in the postmortem brains of all mutant mouse strains than in the corresponding controls (*Shn2* KO, 2.98 ± 0.080 mM, Con, 2.55 ± 0.076 mM, $P=0.015$; *Cn* KO, 3.24 ± 0.051 mM, Con, 2.90 ± 0.073 mM, $P=0.015$; *Nrgn* KO, 2.98 ± 0.11 mM, Con, 2.58 ± 0.054 mM, $P=0.015$; *Camk2a* HKO, 2.86 ± 0.024 mM, Con, 2.58 ± 0.037 mM, $P=0.0012$; *Chd8* HKO, 3.04 ± 0.081 mM, Con, 2.58 ± 0.086 mM, $P=0.015$; Figure 2b). *Z*-score-based analysis revealed a significant negative correlation between pH and lactate levels (Pearson's $r=-0.65$, $P=1.19\times10^{-7}$; Figure 2c). In addition, lactate levels exhibited a significant negative correlation with age in the control group, but not in the mutant group (Supplementary Figure S4). However, there were no significant differences in age between controls and mutants of each strain (see the Materials and Methods). No significant correlations between age and pH were observed in either the mutant or control groups (Supplementary Figure S4).

Disc1-L100P mutant, *Disc1-Q31L* mutant, and *Barp* KO mice do not exhibit behavioral phenotypes associated with psychiatric disorders (Nakao *et al*, 2015; Shoji *et al*, 2012) (Supplementary Table S2). Unlike other model mice, mice of these three lines exhibited no changes in brain pH or lactate levels relative to those observed in the corresponding

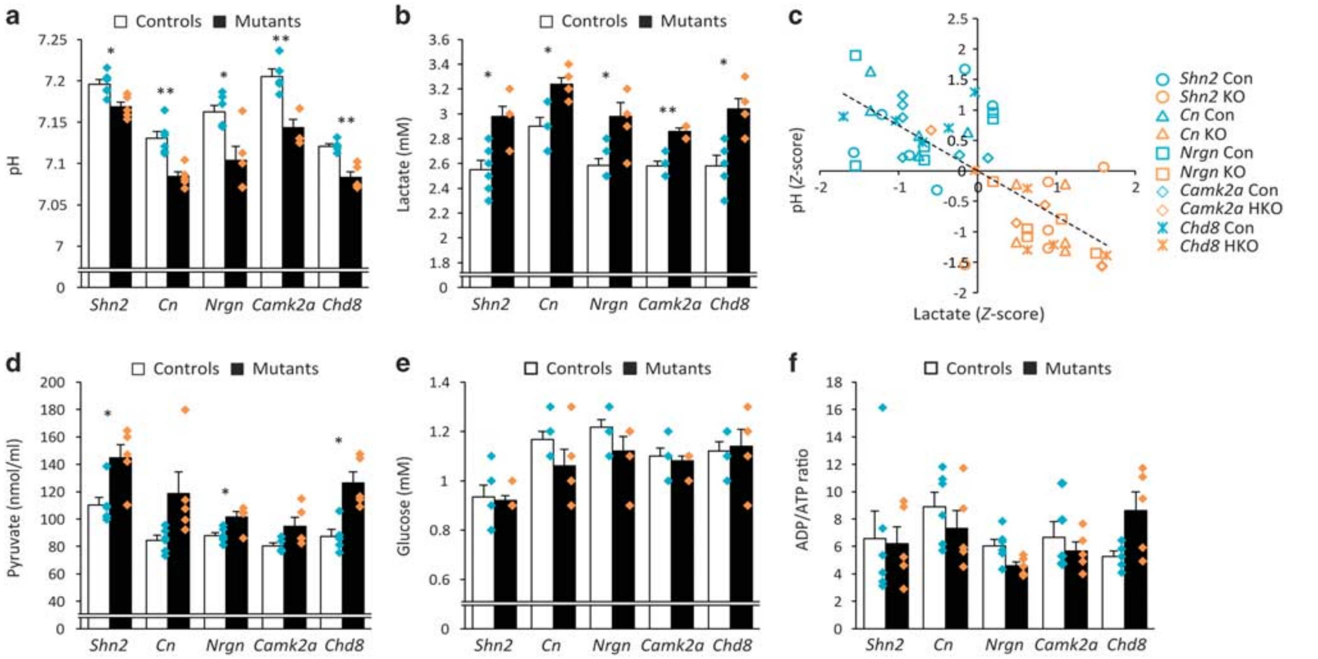

Figure 2 Lower pH and increased lactate levels in the brains of mouse models of psychiatric disorders. Bar graphs of pH (a), lactate levels (b), pyruvate levels (d), glucose levels (e), and ADP/ATP ratio (f) in the brains of *Shn2* KO, *Cn* KO, *Nrgn* KO, *Camk2a* HKO, and *Chd8* HKO mice and their corresponding controls (mean ± SEM). Each plot represents individual mouse values. (c) Scatter plot showing correlations between pH and lactate levels in the mouse brain. Asterisks indicate statistically significant differences between controls and mutants after Bonferroni–Holm correction (*$P<0.05$, **$P<0.01$). ADP, adenosine diphosphate; ATP, adenosine triphosphate.

controls (Supplementary Figure S5). These results suggest that genetic alterations in general do not necessarily cause changes in pH and lactate levels in the mouse brain.

Lactate is formed from pyruvate during glycolysis. We therefore measured pyruvate levels in model mouse brains, observing that levels were significantly increased in *Shn2* KO ($P=0.042$), *Nrgn* KO ($P=0.042$), and *Chd8* HKO mice ($P=0.042$). *Cn* KO ($P=0.092$) and *Camk2a* HKO mice ($P=0.092$) exhibited a trend toward increased pyruvate levels, although this trend did not survive Bonferroni–Holm correction (Figure 2d). Glucose levels remained unchanged in mutant mice relative to controls (Figure 2e), suggesting that glucose supply/demand ratio in the brain may be comparable in these mouse models. In addition, no significant differences in ADP/ATP ratio were observed in these model mice following Bonferroni–Holm correction (Figure 2f), suggesting no alteration of energy consumption ratio in the brains of model mice.

We then analyzed transcriptome data (Supplementary Table S3) in order to investigate the potential underlying molecular mechanisms of increased lactate levels in mutant mouse brains. Transcriptome data from five mouse strains revealed an enrichment in Wnt- and epidermal growth factor (EGF)-related pathways when analyzed using DAVID software (Supplementary Table S4). Enrichment in Wnt-related pathways was replicated in analyses performed using other bioinformatics tools (ADGO and GOToolBox) using different statistical methods (Supplementary Table S4).

As lactate is produced via glycolytic pathways in astrocytes in the brain (Demetrius and Simon, 2012), we analyzed the transcriptome data of model mice with particular focus on glycolysis-related genes (Gene Ontology Consortium database) as well as those related to pyruvate metabolism. The results of the targeted gene expression analyses suggest that elevated glycolysis and pyruvate metabolism shifting toward lactate synthesis occur in the brains of model mice, especially in *Shn2* KO and *Camk2a* HKO mice (Supplementary Table S5 and Supplementary Figure S6).

DISCUSSION

In the present study, our meta-analysis confirmed that the brains of patients with schizophrenia and bipolar disorder exhibit lower pH than healthy controls upon postmortem examination. Lower pH was also observed in five different mouse models of psychiatric disorders, all of which were drug naive and controlled for other potential confounding factors, such as agonal state and postmortem interval. We also observed increased lactate levels in the brains of model mice, as well as a highly significant negative correlation between pH and lactate levels, consistent with the findings of previous human postmortem studies (Halim *et al*, 2008). These results suggest that lower pH and increased lactate levels are implicated in the underlying pathophysiology of the diseases rather than mere artifacts.

Our meta-analyses indicated that brain pH is decreased in patients with schizophrenia and bipolar disorder when postmortem interval, age, and antipsychotic use are regarded as potential covariates of brain pH. There are, however, still limitations regarding the covariates in the present study. One major limitation involves the lack of data regarding agonal state, as previous studies have reported that individuals who experience prolonged agonal states exhibit lower brain pH (Mexal *et al*, 2006; Tomita *et al*, 2004). These findings suggest that agonal state may be a potential confounding factor for postmortem brain pH. As such, studies using animal models are necessary to validate the findings from human postmortem studies. However, as we cannot rule out the possibility that agonal states were altered in our model mice, further studies should examine pH and lactate levels in the brains of animals with short, moderate, and prolonged agonal states.

In addition to these limitations, other secondary factors cannot be ruled out in animal studies. For example, a recent study using *Tbx1* mutant mice (ASD model mice) revealed that a genetic risk factor can influence maternal care via the phenotype of the risk carrier (Takahashi *et al*, 2016). Such secondary environmental factors during childhood may thus affect brain pH in mice. Increased locomotor activity may alter oxygen levels in the brains, particularly in *Shn2* KO, *Cn* KO, *Nrgn* KO, and *Camk2a* HKO mice (Supplementary Table S2). Considering that blood oxygen levels are positively correlated with brain lactate levels (Bednařík *et al*, 2015), oxygen levels may also represent a potential confounding factor in the measurement of brain lactate levels. Moreover, the balance between blood and brain cells may differ between the brain homogenates of control and mutant mice. As lactate concentrations in the blood are two- to three-fold higher than those in the mouse brain (extracellular compartment) (Béland-Millar *et al*, 2017), such differences may represent an additional confounding factor. Although differences in genetic background are also of concern when making comparisons between different mouse strains (Wolfer *et al*, 2002), we utilized over nine backcrossings in addition to littermates within each strain. Thus, it is unlikely that differences in pH and lactate levels between control and mutant mice within each strain reflect differences in genetic background.

We observed no significant differences in pH and lactate levels in the brain of two lines of *Disc1* mutant mice as compared with corresponding controls. *DISC1* has been implicated in the genetic etiology of schizophrenia (Brandon and Sawa, 2011; Ishizuka *et al*, 2006). However, a recent large-scale analysis of copy number variants (CNVs) suggested that *DISC1* may not be a risk factor for the disorder (Marshall *et al*, 2016); the analysis showed that there are 5 deletions and 1 duplication in 21 094 schizophrenia cases and 4 deletions and 2 duplications in 20 227 controls (data deposited at UCSC Genome Browser on Human Mar. 2006, NCBI36/hg18). These results, combined with our previous observations that *Disc1* mutant mice used in the present study did not exhibit behavioral phenotypes associated with schizophrenia (Shoji *et al*, 2012), suggest that these mutant mice may not represent a valid animal model of the disorder.

Increased lactate levels have been observed within certain brain regions in patients with schizophrenia (Halim *et al*, 2008; Prabakaran *et al*, 2004), bipolar disorder (Dager *et al*, 2004; Lan *et al*, 2008), and ASD (Goh *et al*, 2014) in both postmortem and *in vivo* spectroscopic imaging studies. More recent studies have confirmed increased lactate levels in the brains of patients with schizophrenia by postmortem analysis (Dean *et al*, 2016) and *in vivo* analysis using 7-Tesla magnetic resonance spectroscopy (MRS) (Rowland *et al*, 2016). Additional studies have revealed that pyruvate levels are increased whereas glucose levels remain unchanged in the postmortem brains of patients with schizophrenia (Dean *et al*, 2016). Our findings in mouse models are substantially consistent with the evidence obtained from previous human studies.

In our animal experiments, control baselines of pH and lactate levels varied among strains (Figures 2a and b). Such variations may have been due to differences in age, as we observed a significant negative correlation between lactate levels and age in control mice; lactate levels increased as mouse age decreased (Supplementary Figure S4). These findings align with the neurodevelopmental hypothesis of schizophrenia. Accumulating evidence suggests that maturation abnormalities in certain types of brain cells, in which the molecular and physiological properties are similar to those of normal immature cells, represent an endophenotype commonly observed in several neuropsychiatric disorders, including schizophrenia, bipolar disorder, and epilepsy (Hagihara *et al*, 2013; Shin *et al*, 2013; Walton *et al*, 2012). For example, our previous study demonstrated that gene expression patterns in the prefrontal cortex of patients with schizophrenia are strikingly similar to those of typically developing infants (Hagihara *et al*, 2014). Considering the negative correlation between age and lactate levels in control mice of the present study, higher lactate levels in mutant mice may reflect one aspect of maturational abnormalities of the brain. However, additional studies involving detailed time-course analyses of developmental changes in pH and lactate levels are required to verify this hypothesis.

Previous studies have also revealed that brain acidosis influences a number of brain functions, such as anxiety, mood, and cognition (Wemmie, 2011). Acidosis may affect the structure and function of several types of brain cells, including the electrophysiological functioning of GABAergic neurons (Huang *et al*, 2015) and morphological properties of oligodendrocytes (Goldman *et al*, 1989). Alterations in these types of cells have been well documented in the brains of patients with schizophrenia, bipolar disorder, and ASD (Bartzokis, 2005; Nakazawa *et al*, 2012) and may underlie some of the cognitive deficits associated with these disorders. Deficits in GABAergic neurons and oligodendrocytes have also been identified in mouse models of the disorders, including *Shn2* KO mice (Takao *et al*, 2013). Brain acidosis may therefore be associated with deficits in such cell types in schizophrenia, bipolar disorder, and ASD. However, as each genetic alteration may dysregulate the neurochemical balances of downstream molecules that are not functionally relevant to psychiatric disorders, further studies are required to determine whether low pH and increased lactate levels are functionally significant in psychiatric disorders.

A previous study indicated that chronic treatment with antipsychotics increases lactate levels in the rat cerebral cortex (Halim *et al*, 2008), 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 in the post-mortem brains of patients with schizophrenia. In addition, increased lactate levels have been observed in the anterior cingulate of medication-free patients with bipolar disorder in *in vivo* spectroscopic imaging studies (Dager *et al*, 2004). Furthermore, studies utilizing animal models of psychiatric disorders have identified increased lactate levels in mutant mouse brains *in vivo* (das Neves Duarte *et al*, 2012). In addition, we observed an association between increased lactate levels and decreased pH in the brains of model mice in the present study, consistent with findings from previous studies on patients with schizophrenia (Halim *et al*, 2008; Prabakaran *et al*, 2004). Decreased brain pH has also been observed in medication-free patients with bipolar disorder (Kato *et al*, 1998). Although it is 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 disorder.

It should be noted that haploinsufficiency of *Chd8*, a gene associated with ASD, results in lower brain pH and increased lactate levels in mice, as well as molecular and behavioral phenotypes relevant to ASD (Katayama *et al*, 2016). Previous studies have reported that patients with ASD exhibit decreased pH (Young *et al*, 2011) and increased lactate levels (Goh *et al*, 2014) relative to healthy controls, suggesting that our findings in model mice are consistent with those observed in patients with ASD. In addition, *CHD8* has also been implicated in schizophrenia (Kimura *et al*, 2016; McCarthy *et al*, 2014). Therefore, lower pH and increased lactate levels in *Chd8* HKO mice may reflect an aspect of brain pathophysiology in ASD/schizophrenia.

Interestingly, we observed that Wnt- and EGF-related pathways, which are highly implicated in somatic and brain cancers (Nicholas *et al*, 2006), are enriched in the genes whose expressions were altered among the five mutant mouse strains. It is well known that cancer cells display high rates of glycolysis, resulting in high lactate and pyruvate levels, even in normoxia (Lu *et al*, 2002). This phenomenon has been referred to as the Warburg effect. Genes whose expression is known to positively regulate the Warburg effect, such as *Hk2* (Mathupala *et al*, 2009), *Hif1a* (Lu *et al*, 2002), and *Pfkfb3* (Minchenko *et al*, 2002), were increased in the brains of some of mouse models examined in the present study, whereas expression of *Prkaa1*—a negative regulator of the Warburg effect (Faubert *et al*, 2013)—was decreased (Supplementary Table S3). These findings suggest that elevated glycolysis underlies increases in lactate and pyruvate levels in the brains of schizophrenia, bipolar disorder, and ASD model mice. The results of the targeted gene expression analyses conducted in the present study also support this hypothesis. Glycolysis is also stimulated by the uptake of glutamate in astrocytes following neuronal excitation (Pellerin and Magistretti, 1994). Dysregulation of the excitation–inhibition balance has been proposed as a candidate cause of schizophrenia, bipolar disorder, and ASD (Brealy *et al*, 2015; Marín, 2012). A shift in the balance toward excitation would result in increased energy expenditure and may lead to increased glycolysis. Indeed, *Shn2* KO mice exhibit higher glutamate levels in the hippocampus (Takao *et al*, 2013). *In vivo* metabolite measurements have suggested that increased glycolysis also occurs in the brains of patients with schizophrenia (Rowland *et al*, 2016) and bipolar disorder (Dager *et al*, 2004; Stork and Renshaw, 2005), whereas gene ontology analysis of microarray data has suggested that decreased glycolysis occurs in the brains of patients with schizophrenia (Prabakaran *et al*, 2004). Although further studies are required to determine whether alterations in the rate of glycolysis are associated with increased lactate levels and decreased pH, we hypothesize that decreased pH in whole-brain samples was due to increased lactate production driven by hyperactivity within specific neural circuits (Hagihara *et al*, 2013; Heckers and Konradi, 2015; Lisman *et al*, 2008). Therefore, it would be of interest to investigate pH and lactate levels, as well as glycolysis rate, in the brains of mouse models of other mental disorders in which such hyperactivity has been implicated, such as epilepsy (Seifert and Steinhäuser, 2013), depression (Grace, 2016), and Alzheimer's disease (Busche *et al*, 2012).

Previous studies have indicated that lactate levels in the mouse brain rapidly increase after at least 1 min of decapitation, relative to those observed following *in vivo* fixation via focused microwave irradiation, regarded as a consequence of enhanced glycolysis under oxygen-deprived conditions (Sugiura *et al*, 2014). Although the current findings may differ from those obtained under physiological conditions, they may also reflect functional changes (eg, astrocyte activation) (Huang and Huang, 2012; Takao *et al*, 2013) that represent the main source of lactate production in the brain.

Brain pH is associated with notable changes in gene expression (Catts *et al*, 2005; Iwamoto *et al*, 2005; Mexal *et al*, 2006; Tomita *et al*, 2004) 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 tissue pH between patients and controls. Given that lower brain pH is a pathophysiological component of certain conditions, pH-dependent changes in gene expression are of concern when attempting to elucidate the molecular basis of the conditions. Some studies have indicated that gene expression patterns are partially similar across diseases such as schizophrenia, bipolar disorder, and ASD (Ellis *et al*, 2016; Shao and Vawter, 2008). Decreased pH may underlie these similarities in the pattern of gene expression. Thus, pH may be an important factor in the elucidation of molecular alternations in the brains of patients with these psychiatric conditions.

FUNDING AND DISCLOSURE

TM received research grants from Astellas Pharma Inc. CSW is on an advisory board for Lundbeck, Australia Pty Ltd and in collaboration with Astellas Pharma Inc. CSW is funded by the NSW Ministry of Health, Office of Health and Medical Research. CSW is a recipient of a National Health and Medical Research Council (Australia) Principal Research Fellowship (PRF) (#1117079). CSW and VSC received tissues from the New South Wales Brain Tissue Resource Centre at the University of Sydney which is supported by the University of Sydney and The Schizophrenia Research Institute (Fillman et al, 2013). Research reported in this publication was supported by the National Institute of Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number R28AA012725. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

ACKNOWLEDGMENTS

We thank Gerald R Crabtree of Stanford University for providing *Cn* mutant mice, Wakako Hasegawa, Yumiko

Mobayashi, Misako Murai, Tamaki Murakami, Miwa Takeuchi, Aki Miyakawa, Chikako Ozeki, Yuya Ouchi, Satoko Hattori, Toshiki Kameyama, Hidehito Inagaki, and Hiroki Kurahashi of Fujita Health University for their technical support in this study, as well as Yuki Sugiura of Keio University for helpful discussion. This work was supported by the Grants-in-Aid for Scientific Research (JP25242078) from Japan Society for the Promotion of Science; the Grant-in-Aid for Scientific Research on Innovative Areas "Unraveling the microendophenotypes of psychiatric disorders at the molecular, cellular and circuit levels" (JP25116526, JP15H01297) and "Dynamic regulation of brain function by Scrap & Build system" (JP16H06462) from the Ministry of Education, Culture, Sports, Science and Technology; and the Strategic Research Program for Brain Sciences from Japan Agency for Medical Research and Development, AMED.

REFERENCES

Bartzokis G (2005). Brain myelination in prevalent neuropsychiatric developmental disorders. *Adolesc Psychiatry* **29**: 55–96.

Bednařík P, Tkáč I, Giove F, DiNuzzo M, Deelchand DK, Emir UE *et al* (2015). Neurochemical and BOLD responses during neuronal activation measured in the human visual cortex at 7 Tesla. *J Cereb Blood Flow Metab* **35**: 601–610.

Béland-Millar A, Larcher J, Courtemanche J, Yuan T, Messier C (2017). Effects of systemic metabolic fuels on glucose and lactate levels in the brain extracellular compartment of the mouse. *Front Neurosci* **11**: 7.

Brandon NJ, Sawa A (2011). Linking neurodevelopmental and synaptic theories of mental illness via DISC1. *Nat Rev Neurosci* **12**: 707–722.

Brealy JA, Shaw A, Richardson H, Singh KD, Muthukumaraswamy SD, Keedwell PA (2015). Increased visual gamma power in schizoaffective bipolar disorder. *Psychol Med* **45**: 783–794.

Busche MA, Chen X, Henning HA, Reichwald J, Staufenbiel M, Sakmann B *et al* (2012). Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* **109**: 8740–8745.

Carroll LS, Owen MJ (2009). Genetic overlap between autism, schizophrenia and bipolar disorder. *Genome Med* **1**: 102.

Catts VS, Catts SV, Fernandez HR, Taylor JM, Coulson EJ, Lutze-Mann LH (2005). A microarray study of post-mortem mRNA degradation in mouse brain tissue. *Mol Brain Res* **138**: 164–177.

Cottrell JR, Levenson JM, Kim SH, Gibson HE, Richardson KA, Sivula M *et al* (2013). Working memory impairment in calcineurin knock-out mice is associated with alterations in synaptic vesicle cycling and disruption of high-frequency synaptic and network activity in prefrontal cortex. *J Neurosci* **33**: 10938–10949.

Cross-Disorder Group of the Psychiatric Genomics Consortium (2013a). Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet* **45**: 984–994.

Cross-Disorder Group of the Psychiatric Genomics Consortium (2013b). Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* **381**: 1371–1379.

Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK *et al* (2004). Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry* **61**: 450–458.

das Neves Duarte JM, Kulak A, Gholam-Razaee MM, Cuenod M, Gruetter R, Do KQ (2012). N-acetylcysteine normalizes neurochemical changes in the glutathione-deficient schizophrenia mouse model during development. *Biol Psychiatry* **71**: 1006–1014.

Dean B, Thomas N, Scarr E, Udawela M (2016). Evidence for impaired glucose metabolism in the striatum, obtained postmortem, from some subjects with schizophrenia. *Transl Psychiatry* **6**: e949.

Demetrius LA, Simon DK (2012). An inverse-Warburg effect and the origin of Alzheimer's disease. *Biogerontology* **13**: 583–594.

Ellis SE, Panitch R, West AB, Arking DE (2016). Transcriptome analysis of cortical tissue reveals shared sets of downregulated genes in autism and schizophrenia. *Transl Psychiatry* **6**: e817.

Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z *et al* (2013). AMPK is a negative regulator of the Warburg Effect and suppresses tumor growth in vivo. *Cell Metab* **17**: 113–124.

Fillman SG, Cloonan N, Catts VS, Miller LC, Wong J, McCrossin T *et al* (2013). Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol Psychiatry* **18**: 206–214.

Goh S, Dong Z, Zhang Y, DiMauro S, Peterson BS (2014). Mitochondrial dysfunction as a neurobiological subtype of autism spectrum disorder: evidence from brain imaging. *JAMA Psychiatry* **71**: 665–671.

Goldman SA, Pulsinelli WA, Clarke WY, Kraig RP, Plum F (1989). The effects of extracellular acidosis on neurons and glia in vitro. *J Cereb Blood Flow Metab* **9**: 471–477.

Goodkind M, Eickhoff SB, Oathes DJ, Jiang Y, Chang A, Jones-Hagata LB *et al* (2015). Identification of a common neurobiological substrate for mental illness. *JAMA Psychiatry* **72**: 305–315.

Grace AA (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nat Rev Neurosci* **17**: 524–532.

Guillozet-Bongaarts AL, Hyde TM, Dalley RA, Hawrylycz MJ, Henry A, Hof PR *et al* (2014). Altered gene expression in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Mol Psychiatry* **19**: 478–485.

Hagihara H, Horikawa T, Nakamura HK, Umemori J, Shoji H, Kamitani Y *et al* (2016). Circadian gene circuitry predicts hyperactive behavior in a mood disorder mouse model. *Cell Rep* **14**: 2784–2796.

Hagihara H, Ohira K, Takao K, Miyakawa T (2014). Transcriptomic evidence for immaturity of the prefrontal cortex in patients with schizophrenia. *Mol Brain* **7**: 41.

Hagihara H, Takao K, Walton NM, Matsumoto M, Miyakawa T (2013). Immature dentate gyrus: an endophenotype of neuropsychiatric disorders. *Neural Plast* **2013**: e318596.

Hagihara H, Toyama K, Yamasaki N, Miyakawa T (2009). Dissection of hippocampal dentate gyrus from adult mouse. *J Vis Exp* **33** pii: 1543.

Halim ND, Lipska BK, Hyde TM, Deep-Soboslay A, Saylor EM, Herman M *et al* (2008). Increased lactate levels and reduced pH in postmortem brains of schizophrenics: medication confounds. *J Neurosci Methods* **169**: 208–213.

Heckers S, Konradi C (2015). GABAergic mechanisms of hippocampal hyperactivity in schizophrenia. *Schizophr Res* **167**: 4–11.

Huang FL, Huang K-P (2012). Methylphenidate improves the behavioral and cognitive deficits of neurogranin knockout mice. *Genes Brain Behav* **11**: 794–805.

Huang FL, Huang K-P, Wu J, Boucheron C (2006). Environmental enrichment enhances neurogranin expression and hippocampal learning and memory but fails to rescue the impairments of neurogranin null mutant mice. *J Neurosci* **26**: 6230–6237.

Huang L, Zhao S, Lu W, Guan S, Zhu Y, Wang J-H (2015). Acidosis-induced dysfunction of cortical GABAergic neurons through astrocyte-related excitotoxicity. *PLoS ONE* **10**: e0140324.

Hyman SE (2010). The diagnosis of mental disorders: the problem of reification. *Annu Rev Clin Psychol* **6**: 155–179.

Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K *et al* (2010). Research domain criteria (RDoC): toward a new

classification framework for research on mental disorders. *Am J Psychiatry* **167**: 748–751.
Ishizuka K, Paek M, Kamiya A, Sawa A (2006). A review of Disrupted-in-Schizophrenia-1 (disc1): neurodevelopment, cognition, and mental conditions. *Biol Psychiatry* **59**: 1189–1197.
Iwamoto K, Bundo M, Kato T (2005). Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Hum Mol Genet* **14**: 241–253.
Katayama Y, Nishiyama M, Shoji H, Ohkawa Y, Kawamura A, Sato T *et al* (2016). CHD8 haploinsufficiency results in autistic-like phenotypes in mice. *Nature* **537**: 675–679.
Kato T, Murashita J, Kamiya A, Shioiri T, Kato N, Inubushi T (1998). Decreased brain intracellular pH measured by 31P-MRS in bipolar disorder: a confirmation in drug-free patients and correlation with white matter hyperintensity. *Eur Arch Psychiatry Clin Neurosci* **248**: 301–306.
Kimura H, Wang C, Ishizuka K, Xing J, Takasaki Y, Kushima I *et al* (2016). Identification of a rare variant in CHD8 that contributes to schizophrenia and autism spectrum disorder susceptibility. *Schizophr Res* **178**: 104–106.
Kraut JA, Madias NE (2014). Lactic acidosis. *N Engl J Med* **371**: 2309–2319.
Lan MJ, McLoughlin GA, Griffin JL, Tsang TM, Huang JTJ, Yuan P *et al* (2008). Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. *Mol Psychiatry* **14**: 269–279.
Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV *et al* (2004). Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. *Hum Mol Genet* **13**: 609–616.
Lipska BK, Deep-Soboslay A, Weickert CS, Hyde TM, Martin CE, Herman MM *et al* (2006). Critical factors in gene expression in postmortem human brain: focus on studies in schizophrenia. *Biol Psychiatry* **60**: 650–658.
Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S *et al* (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* **31**: 234–242.
Lotan A, Fenckova M, Bralten J, Alttoa A, Dixson L, Williams RW *et al* (2014). Neuroinformatic analyses of common and distinct genetic components associated with major neuropsychiatric disorders. *Front Neurosci* **8**: 331.
Lu H, Forbes RA, Verma A (2002). Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem* **277**: 23111–23115.
Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS *et al* (2016). Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. *Nat Genet* **49**: 27–35.
Marín O (2012). Interneuron dysfunction in psychiatric disorders. *Nat Rev Neurosci* **13**: 107–120.
Mathupala SP, Ko YH, Pedersen PL (2009). Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg effect" and a pivotal target for effective therapy. *Semin Cancer Biol* **19**: 17–24.
McCarthy SE, Gillis J, Kramer M, Lihm J, Yoon S, Berstein Y *et al* (2014). De novo mutations in schizophrenia implicate chromatin remodeling and support a genetic overlap with autism and intellectual disability. *Mol Psychiatry* **19**: 652–658.
Mexal S, Berger R, Adams CE, Ross RG, Freedman R, Leonard S (2006). Brain pH has a significant impact on human postmortem hippocampal gene expression profiles. *Brain Res* **1106**: 1–11.
Minchenko A, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead V *et al* (2002). Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect. *J Biol Chem* **277**: 6183–6187.
Mistry M, Gillis J, Pavlidis P (2013). Meta-analysis of gene coexpression networks in the post-mortem prefrontal cortex of patients with schizophrenia and unaffected controls. *BMC Neurosci* **14**: 105.
Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H *et al* (2003). Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proc Natl Acad Sci USA* **100**: 8987–8992.
Nakao A, Miki T, Shoji H, Nishi M, Takeshima H, Miyakawa T *et al* (2015). Comprehensive behavioral analysis of voltage-gated calcium channel beta-anchoring and -regulatory protein knockout mice. *Front Behav Neurosci* **9**: 141.
Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S *et al* (2012). GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology* **62**: 1574–1583.
Narins RG, Emmett M (1980). Simple and mixed acid-base disorders: a practical approach. *Medicine (Baltimore)* **59**: 161–187.
Nicholas MK, Lukas RV, Jafri NF, Faoro L, Salgia R (2006). Epidermal growth factor receptor–mediated signal transduction in the development and therapy of gliomas. *Am Assoc Cancer Res* **12**: 7261–7270.
Pak JH, Huang FL, Li J, Balschun D, Reymann KG, Chiang C *et al* (2000). Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: a study with knockout mice. *Proc Natl Acad Sci USA* **97**: 11232–11237.
Pellerin L, Magistretti PJ (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* **91**: 10625–10629.
Posner JB, Plum F (1967). Spinal-fluid pH and neurologic symptoms in systemic acidosis. *N Engl J Med* **277**: 605–613.
Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT-J, Griffin JL *et al* (2004). Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry* **9**: 684–697 643.
Rowland LM, Pradhan S, Korenic S, Wijtenburg SA, Hong LE, Edden RA *et al* (2016). Elevated brain lactate in schizophrenia: a 7 T magnetic resonance spectroscopy study. *Transl Psychiatry* **6**: e967.
Ryan MM, Lockstone HE, Huffaker SJ, Wayland MT, Webster MJ, Bahn S (2006). Gene expression analysis of bipolar disorder reveals downregulation of the ubiquitin cycle and alterations in synaptic genes. *Mol Psychiatry* **11**: 965–978.
Seifert G, Steinhäuser C (2013). Neuron–astrocyte signaling and epilepsy. *Exp Neurol* **244**: 4–10.
Shao L, Vawter MP (2008). Shared gene expression alterations in schizophrenia and bipolar disorder. *Biol Psychiatry* **64**: 89–97.
Shin R, Kobayashi K, Hagihara H, Kogan JH, Miyake S, Tajinda K *et al* (2013). The immature dentate gyrus represents a shared phenotype of mouse models of epilepsy and psychiatric disease. *Bipolar Disord* **15**: 405–421.
Shoji H, Toyama K, Takamiya Y, Wakana S, Gondo Y, Miyakawa T (2012). Comprehensive behavioral analysis of ENU-induced Disc1-Q31L and -L100P mutant mice. *BMC Res Notes* **5**: 108.
Stork C, Renshaw PF (2005). Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol Psychiatry* **10**: 900–919.
Sugiura Y, Honda K, Kajimura M, Suematsu M (2014). Visualization and quantification of cerebral metabolic fluxes of glucose in awake mice. *Proteomics* **14**: 829–838.
Suh J, Foster DJ, Davoudi H, Wilson MA, Tonegawa S (2013). Impaired hippocampal ripple-associated replay in a mouse model of schizophrenia. *Neuron* **80**: 484–493.
Sun X, Wang J-F, Tseng M, Young LT (2006). Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *J Psychiatry Neurosci* **31**: 189–196.

Takahashi T, Okabe S, Broin PÓ, Nishi A, Ye K, Beckert MV *et al* (2016). Structure and function of neonatal social communication in a genetic mouse model of autism. *Mol Psychiatry* **21**: 1208–1214.
Takao K, Kobayashi K, Hagihara H, Ohira K, Shoji H, Hattori S *et al* (2013). Deficiency of Schnurri-2, an MHC enhancer binding protein, induces mild chronic inflammation in the brain and confers molecular, neuronal, and behavioral phenotypes related to schizophrenia. *Neuropsychopharmacology* **38**: 1409–1425.
Tomita H, Vawter MP, Walsh DM, Evans SJ, Choudary PV, Li J *et al* (2004). Effect of agonal and postmortem factors on gene expression profile: quality control in microarray analyses of postmortem human brain. *Biol Psychiatry* **55**: 346–352.
Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB (2005). Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biol Psychiatry* **57**: 252–260.
Vawter M, Tomita H, Meng F, Bolstad B, Li J, Evans S *et al* (2006). Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. *Mol Psychiatry* **11**: 615–679.
Walton NM, Zhou Y, Kogan JH, Shin R, Webster M, Gross AK *et al* (2012). Detection of an immature dentate gyrus feature in human schizophrenia/bipolar patients. *Transl Psychiatry* **2**: e135.
Wemmie JA (2011). Neurobiology of panic and pH chemosensation in the brain. *Dialogues Clin Neurosci* **13**: 475–483.
Wolfer DP, Crusio WE, Lipp H-P (2002). Knockout mice: simple solutions to the problems of genetic background and flanking genes. *Trends Neurosci* **25**: 336–340.
Yahata N, Morimoto J, Hashimoto R, Lisi G, Shibata K, Kawakubo Y *et al* (2016). A small number of abnormal brain connections predicts adult autism spectrum disorder. *Nat Commun* **7**: 11254.
Yamasaki N, Maekawa M, Kobayashi K, Kajii Y, Maeda J, Soma M *et al* (2008). Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric disorders. *Mol Brain* **1**: 6.
Young A, Campbell E, Lynch S, Suckling J, Powis S (2011). Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation. *Front Psychiatry* **2**: 27.
Zeng H, Chattarji S, Barbarosie M, Rondi-Reig L, Philpot BD, Miyakawa T *et al* (2001). Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and working/episodic-like memory. *Cell* **107**: 617–629.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)