Selective breeding for helplessness in rats alters the metabolic profile of the hippocampus and frontal cortex: a $^1$H-MRS study at 9.4 T

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Abstract

In humans metabolic changes, particularly in frontal areas of the brain, accompany depressive disorders, but few studies were conducted in animal models of depression. We used hydrogen-1 magnetic resonance spectroscopy at 9.4 T to measure the metabolic profiles of the hippocampus and frontal cortex in congenital learned helpless (cLH) and wild-type (WT) rats. The learned helplessness model of depression exposes animals to uncontrollable stress to induce changes in emotion, cognition and behaviour, but cLH rats were selectively bred to show changes in behaviour even without exposure to uncontrollable stress. Experimentally naive male 8- to 10-wk-old cLH ($n=10$) and WT rats ($n=22$) underwent spectroscopy and were exposed to uncontrollable stress 1 wk after the scan. We found that cLH compared to WT rats had lower levels of glutamate in the hippocampus and lower levels of choline-containing compounds in the hippocampus and frontal cortex, but higher levels of taurine and phosphocreatine in these regions, pointing to compensatory efforts of the brain to reduce excitotoxic potential and to increase neuroprotection and energy, possibly as a result of cellular stress and damage. The reduction in choline-containing phospholipids might represent a source or correlate of such stress. Overall, the results indicate that metabolic abnormalities are present in animals with a predisposition to helplessness even without exposure to explicit stress and may help identify non-invasive biomarkers in individuals who are prone to depression.

Received 25 August 2011; Reviewed 14 October 2011; Revised 14 December 2011; Accepted 17 December 2011; First published online 25 January 2012

Key words: Depression, energy metabolism, learned helplessness, proton spectroscopy.

Introduction

Depression is a common psychiatric disorder that has a major impact on people’s lives and productivity. Depression is not a unitary disorder and available antidepressants are not equally effective in all patients or are not effective at all in some patients (Nestler et al. 2002). The discovery of new treatments will depend on our understanding of the neurobiological basis of the disease.

The learned helplessness model of depression assumes that exposure to uncontrollable stress results in learning that an outcome is unrelated to behavioural responding (Peterson et al. 1993; Seligman, 1975). As a consequence, the subject remains passive and refrains from responding even if it can control the outcome, a core symptom of helplessness. But helplessness is not invariably the consequence of uncontrollable stress, suggesting that genetic and/or environmental factors can predispose individuals to depression (Wieland et al. 1986).

To be able to study the effects of a predisposition, Henn and colleagues generated the congenital learned helpless (cLH) and non-helpless (cNLH) rat strains. They were developed by selective breeding of wild-type (WT) Sprague–Dawley rats that either behaved passively following exposure to uncontrollable stress or actively by terminating a foot shock (Henn & Vollmayr, 2005). The cLH strain exhibits characteristics that could indicate a depressive phenotype. Compared to cNLH and/or WT controls, cLH rats more often failed to terminate a foot shock (Henn & Vollmayr, 2005; Schulz et al. 2010), consumed less of a
palatable solution following stress (Enkel et al. 2010), showed a reduced persistence to work for reward (Vollmayr et al. 2004), reduced exploration more over trials (Schulz et al. 2010) and more rapidly acquired and took longer to extinguish a conditioned freezing response (Shumake et al. 2005). More fear-like behaviour was predictive of more severe helplessness in cLH rats (Schulz et al. 2010), consistent with the known relationship between depression and anxiety in humans (Mineka et al. 1998).

In the present study, we used hydrogen-1 magnetic resonance spectroscopy (1H-MRS) to search for biomarkers that could reflect a predisposition to helplessness before any experience of uncontrollable stress. 1H-MRS is a non-invasive in vivo imaging technique that is also used in humans to examine the metabolic profiles of biological systems and thus has immediate translational value. Clinical magnetic resonance scanners have field strengths that usually range between 1.5 and 3.0 T and have a lower spectroscopic resolution than high field scanners, but high field strengths up to 9.4 T are increasingly used in humans (Shajan et al. 2011; Yuh et al. 2006). We employed 1H-MRS at 9.4 T to assess the metabolic profiles of the hippocampal and frontal cortex (FC) in cLH and control rats. The hippocampus is involved in the formation of declarative and episodic memories (Dere et al. 2006; DeVito & Eichenbaum, 2010; Morris et al. 1982; Tulving & Markowitsch, 1998), whereas frontal cortical regions play an important role in working memory (Khan & Muly, 2011). Structural and functional changes in these regions have been found in depressive disorders (Duman & Monteggia, 2006; Nestler et al. 2002) and may be caused by several factors, including a defect in energy metabolism and glutamate (Glu) excitotoxicity (Beal, 1995; Olney et al. 1997). 1H-MRS captures some aspects of energy metabolism that can be used to identify abnormalities in depressive disorders (e.g. for review, see Ende et al. 2006). Although results so far are mixed, in humans the most consistent findings include a reduction in anterior cingulate Glu and glutamine (Glx – hybrid spectrum) and a change in choline-containing compounds (Ende et al. 2006). Few studies have been conducted in animal models of depression, but changes in Glu (Hui et al. 2010; Kim et al. 2010; Li et al. 2008), Glx (Li et al. 2008), choline (Hong et al. 2007, 2009; Hui et al. 2010) and in the ratio of Glu:γ-aminobutyric acid (GABA) (Sartorius et al. 2007) have been reported. The reduction in Glx, if due to Glu, is surprising given that hyperexcitation of Glu neurons might underlie depression (for review, see Tokita et al. 2011) and that Glu receptor blockade produces antidepressant effects (Berman et al. 2000; Trullas & Skolnick, 1990; Yilmaz et al. 2002; but see Bechtholt-Gompf et al. 2011; Popik et al. 2008). Because our scans were conducted at 9.4 T we could resolve the Glx resonance into its two components and also gather information about additional resonances that have not yet been systematically studied with 1H-MRS in relation to depression. To examine whether individual differences in metabolite concentrations could predict the behavioural outcome in a helplessness test, we exposed the rats to uncontrollable stress 1 wk after the scan and used the behavioural responses to controllable stress the next day for correlation with the imaging data.

**Method**

**Subjects**

The protocol for selective breeding of the congenital rat strains is described in detail elsewhere (Henn & Vollmayr, 2005; Schulz et al. 2010). In the present study, we used male, cLH (n = 10) rats, aged 8–10 wk, from our breeding colony at Brookhaven National Laboratory (Schulz et al. 2010). WT Sprague–Dawley (n = 22) rats were purchased from Taconic Farms (USA). On the day of the MRS scan, the cLH and WT rats weighed (g ± S.E.M.) on average 353 ± 5.9 and 371 ± 3.6, respectively. The animals were housed individually (cLH, n = 1) or in groups of two to three in standard polycarbonate cages and were maintained on a 12-h light/dark cycle (lights on 07.00 hours) with free access to food and water. All procedures were approved by Brookhaven National Laboratory’s Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

**1H-MRS**

Anaesthesia was induced with 3–4% of isoflurane gas followed by pentobarbital (40 mg/kg i.p. Nembutal). Glycopyrolate (0.1–0.2 ml) and saline (2 ml) were given together with Nembutal to reduce salivation and for hydration, respectively. The animal was placed supine in a custom-made body holder with the head positioned on a 3 cm diameter radio frequency surface coil. While inside the bore of the magnet, anaesthesia was maintained by a mixture of oxygen and isoflurane gas (up to 2%). The animals’ vital signs (respiration, heart rate and temperature) were monitored throughout the scans using an optical monitoring system (SA Instruments Inc., USA). Imaging was performed on a 9.4 T Biospec Avance 94/20AS MRI scanner (Bruker BioSpin MRI GmbH, Germany). Coronal, axial and sagittal
T2-weighted images were obtained with RARE sequences. These images were later used for anatomical reference when point resolved spectroscopic (PRESS) sequences (1024 averages; imaging time was 34.16 min) were run to assess the metabolic profiles of the left and right hippocampus (LHipp and RHipp, respectively), FC, which included the cingulate and motor cortex, and prefrontal cortex (PFC), which contained the prelimbic and infralimbic cortex as well as anterior portions of cingulate cortex. Each voxel area was $4.5 \times 2 \times 2.5$ mm in size. Examples of voxel placement for each brain region are shown in Fig. 1.

The spectra were acquired using an echo time of 20 ms, a repetition time of 2000 ms and 2048 data points extending over a spectral width of 10.01 parts per million (ppm) or 4006.41 Hz. The respective brain areas were shimmed using MRS Fastmap with a voxel area of 6 mm$^3$. Water suppression was carried out before each PRESS sequence by automated application of a 250 Hz Vapor pulse. The retro frequency lock was turned on for drift correction. The resulting chemical shifts (0.2–4.0 ppm) were analysed with LCModel (Version 6.2; S. W. Provencher). LCModel uses prior information from a linear combination of

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Fig. 1. Voxel areas for the hippocampus, frontal and prefrontal cortex. Examples are shown of voxels placed in (a, b) the right hippocampus, (c, d) the frontal and (e, f) prefrontal cortex. The images are T2-weighted (a) coronal, (b, c, e) axial and (d, f) sagittal slices that were acquired with RARE sequences at 9.4 T.
model spectra to automatically fit and analyse in vivo spectra (Provencher, 2001). The following simulated metabolites were included in the basis set: l-alanine (Ala); aspartate (Asp); creatine (Cr), phosphocreatine (PCr), GABA, glucose (Glc), glutamine (Gln), Glu, glycerophosphocholine (GPC), phosphocholine (PCh), myo-inositol (Ins), l-lactate (Lac), N-acetylaspartate (NAA), N-acetylaspartyl glutamate (NAAG), scyllo-inositol (Scyllo), taurine (Tau), the compounds total choline (tCho = GPC + PCh), total NAA (tNAA = NAA + NAAG), total Cr (tCr = Cr + PCr), and Glu + Glc. The simulated macromolecules were also included. An example of a spectrum that we generated with LCModel is shown in Fig. 2.

For data analysis, relative metabolite concentrations were calculated by taking the ratios of a particular metabolite (mM) over tNAA and tCr. Tissue segmentation into white matter, grey matter and cerebrospinal fluid was not performed. Cramér-Rao lower bounds or estimated standard deviations (%S.D.) ≥ 20, signal:noise (S/N) ratios < 8 and distorted baselines were used as exclusion criteria.

**Learned helplessness**

All animals underwent learned helplessness training and testing 1 wk after the MRS scan. Details of the procedure are published elsewhere (Schulz et al., 2010). In short, Skinner boxes (Coulbourn Instruments, USA) with grid floors were employed to deliver 120 uncontrollable foot shocks at 0.4 mA. Shock durations and inter-shock intervals (ISIs) were randomly varied between 5 and 15 s. The next day, the animals were tested for helplessness. Each box was equipped with a lever and a signal light on top of the lever. The lights turned on and off concurrently with the foot shock. Altogether, 15 foot shocks were delivered, which lasted 60 s unless turned off earlier by one lever press. ISIs were 24 s long. Lever presses were automatically recorded by Graphic State software (Coulbourn Instruments). The training and test sessions took place between 09:00 and 14:00 hours. For each animal, we analysed the number of lever presses that turned off the foot shock within 20 s of shock onset. Animals were considered helpless (LH) when they turned off the foot shock on five or fewer trials (Vollmayr & Henn, 2001) and non-helpless (non-LH) when they pressed the lever six or more times.

**Statistical analysis**

All data used for statistical evaluations were checked for normality and equal variance of the distributions,
using the Shapiro–Wilks test and Levene statistic, respectively. Results did not allow for parametric testing to be used in all cases. Accordingly, group comparisons were performed using the Mann–Whitney U test. For within-group comparisons Wilcoxon’s tests were applied. Spearman’s rank-order correlation procedures were used to examine the relationships between variables. Due to the large number of tests performed, \( p \) values \( < 0.05 \) are presented solely as measures of effect, taking into account the probability of occurrence of type I error.

Results

**S/N ratio**

Mean (± S.E.M.) S/N ratios for each brain area are shown in Fig. 3. The ratio was higher for RHipp than LHipp (cLH: \( p = 0.02 \); WT: \( p = 0.002 \); Wilcoxon’s test) and it was lower for PFC than for any other brain area (cLH: all \( p < 0.01 \); WT: all \( p = 0.008 \)). Moreover, the S/N ratio in PFC (but not in the other areas; all \( p > 0.05 \)) was lower in cLH compared to WT rats (\( p = 0.04 \); Mann–Whitney test). Few data points remained for analysis of PFC once the exclusion criterion of S/N < 8 was applied (see percentages indicated in Fig. 3), so that this brain area was not further analysed.

**Estimated standard errors**

Mean (± S.E.M.) fitting errors (% S.D.) for selected metabolites are shown in Table 1. For these metabolites (or their relative concentrations) group comparisons were carried out.

The remaining resonances (Ala, Asp, Glc, GPC, Lac, Scylo) were poorly resolved with fitting errors \( \geq 20 \) for most animals. Table 1 also shows the number of rats for which the final metabolite ratios were calculated after the exclusion criteria were applied.

**Chemical shift analyses of hippocampus and FC**

Metabolite concentrations relative to tNAA

When the metabolite concentrations were computed relative to tNAA, group differences were found for Glu (Fig. 4c) in LHipp (\( p = 0.05 \)) and RHipp (\( p = 0.03 \)), but not in FC (\( p = 0.64 \)); cLH rats had lower levels of Glu in LHipp and RHipp than WT rats. Moreover, cLH compared to WT rats had lower concentrations of tCho in LHipp (\( p = 0.002 \)) and RHipp (\( p = 0.04 \)), but not in FC (\( p = 0.07 \) (Fig. 4b)).

Group differences for one of the three brain regions were obtained for Cr; cLH compared to WT rats had lower concentrations of Cr in RHipp (\( p = 0.05 \)), but not in LHipp (\( p = 0.08 \) or FC (\( p = 0.17 \)). Moreover, cLH rats had higher concentrations of PCr in LHipp (\( p = 0.05 \)), but not in RHipp (\( p = 0.60 \) or FC (\( p = 0.06 \)). Higher levels of Tau in cLH rats were found in LHipp (\( p = 0.01 \), but not in RHipp (\( p = 0.14 \) or FC (\( p = 0.28 \)). Group differences were not found for GABA, Gln, PCh, Ins, NAA or the compounds tCr and Glu + Gln in any of the brain regions (all \( p > 0.05 \)).
Metabolite concentrations relative to tCr

When tCr was used in the ratio, cLH compared to WT rats exhibited higher concentrations of PCr (LHipp: \( p = 0.04 \); RHipp: \( p = 0.05 \); FC: 0.04; Fig. 5a) and Tau (LHipp: \( p = 0.009 \); RHipp: \( p = 0.01 \); FC: 0.02; Fig. 5b) in all three brain regions. tCho was again lower in cLH compared to WT rats, this time in all three brain regions (LHipp: \( p = 0.005 \); RHipp: \( p = 0.01 \); FC: \( p = 0.02 \); Fig. 5c).

Group differences for one of the three brain regions were obtained for Cr; cLH rats had lower concentrations of Cr in RHipp (\( p = 0.03 \)), but not in LHipp (\( p = 0.06 \)) or FC (\( p = 0.17 \)). Moreover, cLH rats had lower concentrations of Glu in RHipp (\( p = 0.03 \)), but not in LHipp (\( p = 0.21 \)) or FC (\( p = 0.70 \)). Group differences were not found for GABA, Gln, PCh, Ins, NAA or the compounds tNAA and Glu + Gln in any of the brain regions (all \( p > 0.05 \)).

Learned helplessness

As expected, cLH rats turned off the foot shock on fewer trials than WT rats (\( p = 0.001 \); Fig. 6a).

All 10 cLH rats were LH by our criterion of \( \leq 5 \) lever presses within 20 s of shock onset. The WT rats split into LH (\( n = 10 \)) and non-LH (\( n = 12 \)) animals (see Fig. 6b for distribution of individual rats).

Metabolite concentrations in relation to learned helplessness outcome

The relatively large group of WT rats allowed us to compare the metabolite ratios of different subgroups of WT rats with the metabolite ratios of the cLH rats. The results are summarized in Supplementary Table S1 (available online). When tCr was used in the ratio, cLH rats exhibited differences in Tau and tCho compared to both LH and non-LH WT rats, consistent with the results reported for all WT rats combined. However, the group differences in Cr and PCr emerged only in comparison to the non-LH rats. When tNAA was used in the ratio, the results were less consistent across brain regions.

Correlation analyses within the WT group showed that fewer lever presses (indicative of helplessness) coincided with a higher concentration of NAA/tCr (\( r = -0.47, p = 0.03 \); Fig. 7a) and tCho/tCr (\( r = -0.47, p = 0.03 \); Fig. 7b) in LHipp and a lower concentration of Tau/tNAA (\( r = 0.50, p = 0.02 \); Fig. 7c) in this region.

Similar correlations were not found for RHipp, however (all \( p > 0.05 \)). In FC, fewer lever presses were predicted by a higher concentration of tCho/tCr (\( r = -0.60, p = 0.02 \); Fig. 7d). The cLH group exhibited a high number of ties and low variability across the

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Table 1. Mean ± S.E.M. fitting error (% S.D.) in congenital learned helpless (cLH) and wild-type (WT) rats for the left (LHipp) and right (RHipp) hippocampus and frontal cortex (FC)

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<th>LHipp</th>
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<td>Cr</td>
<td>13.33 ± 1.33 (6)</td>
<td>10.65 ± 0.63 (20)</td>
<td>11.25 ± 0.92 (8)</td>
<td>9.10 ± 0.40 (20)</td>
<td>13.50 ± 2.22 (4)</td>
<td>8.57 ± 0.43 (14)</td>
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<td>PCr</td>
<td>7.57 ± 0.43 (7)</td>
<td>7.48 ± 0.27 (21)</td>
<td>7.00 ± 0.41 (9)</td>
<td>6.86 ± 0.37 (22)</td>
<td>8.67 ± 0.88 (6)</td>
<td>7.07 ± 0.46 (15)</td>
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<td>GABA</td>
<td>12.14 ± 0.40 (7)</td>
<td>12.35 ± 0.64 (20)</td>
<td>11.88 ± 0.95 (8)</td>
<td>11.71 ± 0.68 (21)</td>
<td>12.25 ± 1.49 (4)</td>
<td>11.36 ± 0.45 (14)</td>
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<td>Gln</td>
<td>11.57 ± 0.78 (7)</td>
<td>13.30 ± 0.53 (20)</td>
<td>11.44 ± 1.06 (9)</td>
<td>12.05 ± 0.42 (22)</td>
<td>12.00 ± 1.51 (6)</td>
<td>10.47 ± 0.41 (15)</td>
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<td>Glu</td>
<td>5.43 ± 0.20 (7)</td>
<td>5.57 ± 0.18 (21)</td>
<td>5.00 ± 0.29 (9)</td>
<td>5.09 ± 0.19 (22)</td>
<td>4.50 ± 0.43 (6)</td>
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<td>PCh</td>
<td>9.20 ± 2.99 (5)</td>
<td>12.36 ± 1.56 (14)</td>
<td>9.67 ± 2.04 (9)</td>
<td>9.35 ± 1.35 (17)</td>
<td>6.00 ± 2.26 (5)</td>
<td>15.17 ± 0.63 (12)</td>
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<td>Ins</td>
<td>4.40 ± 0.24 (7)</td>
<td>4.19 ± 0.13 (21)</td>
<td>3.56 ± 0.18 (9)</td>
<td>3.82 ± 0.13 (22)</td>
<td>4.67 ± 0.49 (6)</td>
<td>4.13 ± 0.09 (15)</td>
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<td>NAA</td>
<td>3.43 ± 0.20 (7)</td>
<td>3.43 ± 0.13 (21)</td>
<td>3.00 ± 0.17 (9)</td>
<td>3.23 ± 0.11 (22)</td>
<td>3.50 ± 0.22 (6)</td>
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<td>14.26 ± 0.71 (19)</td>
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<td>Tau</td>
<td>4.29 ± 0.18 (7)</td>
<td>5.38 ± 0.15 (21)</td>
<td>4.22 ± 0.15 (9)</td>
<td>4.77 ± 0.20 (22)</td>
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<td>3.89 ± 0.20 (9)</td>
<td>3.82 ± 0.13 (22)</td>
<td>4.00 ± 0.26 (6)</td>
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<tr>
<td>tNAA</td>
<td>3.60 ± 0.24 (7)</td>
<td>3.14 ± 0.08 (21)</td>
<td>2.89 ± 0.20 (9)</td>
<td>2.95 ± 0.12 (22)</td>
<td>3.00 ± 0.26 (6)</td>
<td>2.67 ± 0.13 (15)</td>
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<td>tCr</td>
<td>2.86 ± 0.14 (7)</td>
<td>2.71 ± 0.10 (21)</td>
<td>2.44 ± 0.18 (9)</td>
<td>2.36 ± 0.10 (22)</td>
<td>2.67 ± 0.21 (6)</td>
<td>2.33 ± 0.13 (15)</td>
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<tr>
<td>Glu + Gln</td>
<td>5.43 ± 0.20 (7)</td>
<td>5.71 ± 0.16 (21)</td>
<td>5.33 ± 0.37 (9)</td>
<td>5.32 ± 0.18 (22)</td>
<td>5.17 ± 0.48 (6)</td>
<td>4.53 ± 0.13 (15)</td>
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Cr, Creatine; PCr, phosphocreatine; GABA, γ-aminobutyric acid; Gln, glutamine; Glu, glutamate; PCh, phosphocholine; Ins, myo-inositol; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; Tau, taurine; tCho, total choline; tNAA, total NAA; tCr, total Cr.

Values shown in parentheses indicate the number of rats (\( n \)) for which the mean ± S.D. (after exclusion according to criterion) and final metabolite concentrations were calculated.
helplessness scores, so that correlation analyses were not performed for this group.

**Discussion**

To summarize, we found that naive cLH compared to WT rats had lower concentrations of Glu/tNAA and tCho/tNAA in the hippocampus. They also had lower concentrations of tCho/tCr in the hippocampus and FC and higher concentrations of Tau/tCr and PCr/tCr in these regions. In naive WT animals, a higher level of NAA/tCr in LHipp and of tCho/tCr in LHipp and FC, and a lower level of Tau/tNAA in LHipp were predictive of helplessness as assessed 1 wk after the scan.

**Lower Glu concentrations in cLH compared to WT rats**

One of the most consistent findings of 1H-MRS studies in patients with major depression is a reduction in Glx or Glu in frontal cortical regions, particularly the anterior cingulate cortex (Auer et al. 2000; Hasler et al. 2007; Merkl et al. 2011; Pfleiderer et al. 2003; Rosenberg et al. 2005), with the largest reductions seen in severely depressed patients (for review, see Capizzano et al. 2007). Few studies have examined the hippocampus. Block et al. (2009) found a reduction in left hippocampal Glx in non-medicated depressed patients. Patients who received antidepressant medication and were not severely depressed did not differ from controls (Milne et al. 2009).

Animal studies have been less consistent. Acute exposure to forced swim stress, an animal model of depression, did not alter Glx in the left dorsolateral (dl) PFC (Hong et al. 2007) or increased Glu in the left dlPFC and hippocampus (Kim et al. 2010). Chronic mild stress increased Glu in the left ventral hippocampus of rats that consumed less sucrose during stress (Delgado y Palacios et al. 2011). By contrast, chronic forced swimming over a period of 14 d led to a reduction of Glu and Glx in the PFC and of Glu in the hippocampus (Li et al. 2008). Early life stress, which may induce a predisposition to psychiatric disorders (Heim & Nemeroff, 2001), also led to a reduction of Glu in the hippocampus (Hui et al. 2010). Consistent with the latter, we found a reduction in hippocampal Glu in selectively bred cLH compared to WT rats. Thus, acute stress, which is known to increase the release of Glu (Lowy et al. 1995; Moghaddam, 1993; Rada et al. 2003; Reznikov et al. 2007), and chronic mild stress increased the Glu resonance in animal models of depression, whereas selective breeding for helplessness and prolonged stress caused by early life and chronic forced swim stress decreased the Glu resonance. However, early life stress was also found to increase hippocampal tCho and to decrease ml, a marker for glial cells (Hui et al. 2010), whereas in the present study we found a decrease in hippocampal tCho and no change in ml. A familial predisposition to depression without history of disease was reported to increase Glu in the occipital/parietal cortex (Taylor et al. 2010), a region we did not assess. However, occipital Glu was also increased in depressed patients without family history of depression (Sanacora et al. 2004) and in formerly

![Fig. 4. Metabolite concentrations relative to total N-acetylaspartate (tNAA). Metabolite concentrations were computed relative to N-acetylaspartate (NAA) plus N-acetylaspartyl-glutamate (NAAG). Mean (± SEM) concentrations are shown of (a) glutamate (Glu) and (b) total choline (tCho = glycerophosphocholine plus phosphocholine) for the left and right hippocampus (LHipp and RHipp, respectively) and frontal cortex (FC) in congenital learned helpless (cLH) and wild-type (WT) rats. cLH rats had lower levels of Glu and tCho in the hippocampus compared to WT rats. (* p < 0.05, between the groups; # p < 0.05 FC compared to LHipp and RHipp, within the group; $ p < 0.05 FC compared to RHipp, within the group).](https://academic.oup.com/ijnp/article-abstract/16/1/199/629035/1.300x300)
depressed but fully recovered patients (Bhagwagar et al. 2007).

In contrast to the human studies, we did not observe a change in frontal cortical Glu due to selective breeding of helplessness. One reason for this could relate to the lower S/N ratios in the frontal regions, which led to the loss of a high number of data points, particularly for the PFC. The PFC was furthest away from the centre of the magnetic field and from the most sensitive zone of the surface coil, which was not large enough to provide equal performance from all voxel areas without repositioning the animals.

Lower choline concentrations in cLH compared to WT rats

The tCho resonance consists mainly of GPC and PCh but also of cytidine diphosphate-choline, free choline and acetylcholine (Boulanger et al. 2000). Choline serves as a precursor for the synthesis of the neurotransmitter acetylcholine, which is important for cognitive functions (Bartus et al. 1982), and as a precursor for PCh, which is used in the synthesis of phosphatidylcholine (PtdCho). PtdCho, in turn, is involved in the formation of cell membranes (Wurtman, 1992). GPC and PCh are also breakdown products of PtdCho and thus a source of choline. A reduction of choline and choline-containing phospholipids can have devastating effects on neurons. With a diminished supply of choline, the synthesis of acetylcholine is given priority over membrane building in order to sustain neurotransmission, causing cell shrinkage and death (Ulus et al. 1989; Wurtman, 1992).

The tCho resonance increases with age (Ende et al. 2000; Jung et al. 2002; Pfefferbaum et al. 1999) and with treatment of benzodiazepines (cited in Auer et al. 2000), antidepressants (Block et al. 2009; Sonawalla et al. 1999) and electroconvulsive shock (Biedermann et al. in press; Ende et al. 2000, 2007; Sartorius et al. 2003). When age was corrected for, hippocampal tCho was lower in depressed than remitted patients and in healthy controls (Ende et al. 2000). However, the remitted patients were taking a tricyclic antidepressant at the time of the study, which could have increased the tCho resonance in this group. On the other hand, the same authors found that medicated depressed patients also had lower levels of tCho in the hippocampus (Ende et al. 2007). Medicated but not severely depressed patients who had multiple clinical episodes in the past had elevated tCho levels in the LHipp (Milne et al. 2009). In frontal cortical regions of adult populations, tCho was reduced (Gruber et al. 2003) or differences were not found (Merkl et al. 2011; Nery

![Fig. 5. Metabolite concentrations relative to total creatine (tCr). Metabolite concentrations were computed relative to creatine (Cr) plus phosphocreatine (PCr). Mean ($\pm$ S.E.M.) concentrations are shown of (a) PCr, (b) taurine (Tau) and (c) total choline (tCho = glycerophosphocholine plus phosphocholine) for the left and right hippocampus (LHipp and RHipp, respectively) and frontal cortex (FC) in congenital learned helpless (cLH) and wild-type (WT) rats. cLH rats had higher levels of PCr and Tau but lower levels of tCho in all three brain regions. (* $p<0.05$, between the groups; $\#$ $p<0.05$ FC compared to LHipp and RHipp, within the group; $^*$ $p<0.05$ FC compared to RHipp in cLH rats, and LHipp compared to RHipp in WT rats, within the group).](https://academic.oup.com/ijnp/article-abstract/16/1/199/629035)
Fig. 6. Learned helplessness. One week after hydrogen-1 magnetic resonance spectroscopy was performed, congenital learned helpless (cLH; n = 10) and wild-type (WT; n = 22) rats were exposed to a learned helplessness paradigm. (a) Depicts the mean (± S.E.M.) number of lever presses that turned off the foot shock within 20 s of shock onset. As expected, cLH rats pressed the lever on fewer trials than WT rats. (b) The distribution of helplessness scores for each group (* p < 0.05, between the groups).

Fig. 7. Correlation between metabolite concentration and learned helplessness outcome. In wild-type rats, the number of lever presses that turned off the foot shock within 20 s of shock onset was correlated with N-acetylaspartate (NAA)/total creatine (tCr), total choline (tCho)/tCr and taurine (Tau)/total NAA (tNAA) in the left hippocampus (LHipp) and with tCho/tCr in the frontal cortex (FC). Fewer lever presses, indicative of helplessness, were predicted by a higher concentration of (a) NAA/tCr and (b) tCho/tCr and a lower concentration of (c) Tau/NAA in LHipp and by a higher concentration of (d) tCho/tCr in FC. The data are indicated as ranks. Rank 1 was always assigned to the lowest score. Regression lines were drawn through the data for illustration.
disproportionate influx of Ca\textsuperscript{2+} acids (EAAs) can disrupt cell functioning through neuroprotective functions and could indicate a neuroprotective effect (Huxtable, 1992). For example, Glu and other excitatory amino acids (EAAs) can disrupt cell functioning through disproporionate influx of Ca\textsuperscript{2+} into the cell and attenuation of mitochondrial energy metabolism (El Idrissi & Trenkner, 1999). Tau, on the other hand, regulates calcium homeostasis by reducing the duration of Ca\textsuperscript{2+} transients and increasing energy metabolism in the presence of EAA (El Idrissi, 2008; El Idrissi & Trenkner, 1999, 2004). An increase in extracellular Tau was found to inhibit the release of Glu and other EAA, thus reducing neurotoxic potential (Olive et al., 2000). Tau is also an important osmoregulator that responds to the swelling and shrinking of cell volume (Lambert, 2004, 2007) and stabilizes cell membranes through interactions with phospholipids (Huxtable, 1992).

At clinical field strength, Tau cannot be resolved with \textsuperscript{1}H-MRS, but increases in blood plasma levels of Tau have been reported in depressed patients (Altamura et al., 1993, 1995; Mauri et al., 1998; Mitani et al., 2006). Whether patients responded to antidepressant treatment or not was unrelated to the levels of Tau; treatment alone was found to decrease Tau (Maes et al., 1998, but see Mauri et al., 1998). It is not clear whether plasma levels of Tau are related to central nervous system Tau, particularly across different brain regions. For example, mice whose diet was supplemented with Tau exhibited elevated plasma levels of Tau and elevated levels of Tau in the hypothalamus but not in the cortex (Murakami & Furuse, 2010). These changes coincided with a reduction of immobility in the forced swim test, indicative of an antidepressant effect. However, care must be taken in interpreting this effect, because Tau supplementation also increased locomotor activity (El Idrissi et al., 2009 but see Murakami & Furuse, 2010), which can confound behaviour in the forced swim test. In an ex vivo \textsuperscript{1}H-MRS study, cortical Tau was reduced following exposure to chronic forced swim stress (Li et al., 2008). In an in vivo \textsuperscript{1}H-MRS study, hippocampal Tau was higher in cLH compared to WT rats that were tested for helplessness shortly before the scans (Biedermann et al. in press). Clearly, more work is needed to accurately relate central Tau to depression-like states and behaviour.

**Higher Tau concentrations in cLH compared to WT rats**

Tau has a wide variety of functions (Huxtable, 1992), which makes it difficult to attribute its increase in LHipp, RHipp and FC of cLH rats to any specific one. However, an increase in Tau is generally associated with neuroprotective functions and could indicate a compensatory effect (Huxtable 1989; Lehmann et al., 1984). For example, Glu and other excitatory amino acids (EAAs) can disrupt cell functioning through disproporionate influx of Ca\textsuperscript{2+} into the cell and attenuation of mitochondrial energy metabolism (El Idrissi & Trenkner, 1999). Tau, on the other hand, regulates calcium homeostasis by reducing the duration of Ca\textsuperscript{2+} transients and increasing energy metabolism in the presence of EAA (El Idrissi, 2008; El Idrissi & Trenkner, 1999, 2004). An increase in extracellular Tau was found to inhibit the release of Glu and other EAA, thus reducing neurotoxic potential (Olive et al., 2000). Tau is also an important osmoregulator that responds to the swelling and shrinking of cell volume (Lambert, 2004, 2007) and stabilizes cell membranes through interactions with phospholipids (Huxtable, 1992).

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**Higher PCr (but lower Cr) concentrations in cLH compared to WT rats**

The concentration of PCr was found to be higher in LHipp, RHipp and FC of cLH compared to WT rats, whereas Cr was lower in RHipp and tended to be lower in LHipp. Adenosine triphosphate (ATP) levels are maintained by replenishment from PCr, a secondary energy source. The concentration of PCr varies with changes in the demand and supply of ATP; increases in demand, e.g. during physical exercise and mental activity, tend to decrease PCr and to increase Cr (for review, see Kemp, 2000). Thus, the higher PCr levels in cLH rats might indicate a tendency to conserve energy or to expend energy differently from normal rats. This might be an adaptive response, since increases in PCr buffer ATP levels under conditions of stress and energy depletion, providing neuroprotection (Matthews et al., 1999).

Few studies have reported a change in Cr in patients with depression. tCr is typically used as the denominator in ratios that assess relative metabolite concentrations, assuming that the values remain relatively stable across groups. Here, we did not find a change in relative concentrations of tCr/tNAA, consistent with another study (Hasler et al., 2007). In one study, however, patients with depression had higher absolute levels of tCr in the frontal lobe (Gruber et al., 2003). In mice, absolute levels of tCr were reduced in dIPFC immediately after exposure to acute forced swim stress (Kim et al., 2010). Chronic social stress in tree shrews led to a reduction in absolute levels of tCr in the hippocampus (Czeh et al., 2001), and in helpless rats hippocampal tCr/tNAA was increased following electroconvulsive shock (Sartorius et al., 2003). None of the studies so far reported any values for PCr and Cr separately, which were up- and down-regulated, respectively, in cLH compared to WT rats. We were able to separate the resonances for PCr and Cr with LCModel software, but note that the estimated
standard deviations were somewhat high for the individual metabolites (see Table 1), so these results should be interpreted with caution.

**Correlation of metabolite concentrations with helplessness**

In WT rats, we found that levels of NAA, tCho and Tau were correlated with the behavioural outcome in the helplessness test. Interestingly, the relationships were opposite to what would be expected based on the group differences alone. For example, cLH rats had lower concentrations of tCho in LHipp, RHipp and FC than WT rats, but within the WT group higher levels of tCho in LHipp and FC predicted helplessness. This is consistent with the finding that, in normal individuals, tCho in the left frontal lobe was negatively correlated with positive affect (Jung et al. 2002). 1H-MRS studies tentatively indicate that metabolic changes are left lateralized in depressive disorders (Capizzano et al. 2007). In the present study, helplessness behaviour was correlated with metabolites in LHipp but not RHipp. The quality of the shims was similar between the left and right sides, indicating that the left vs. right differences were not caused by methodological errors, such as the positioning of the animals.

The metabolites were measured 1 wk before the behavioural test, so they are unlikely to reflect the substrates of lever-pressing behaviour per se. Rather, they may reflect a general propensity or baseline neurochemical state that is capable of influencing a behavioural outcome. This baseline state might differ from the predisposition we presume to exist in cLH rats because these rats exhibited differences in metabolite levels compared to both helpless and non-helpless WT rats, at least with regard to Tau and tCho, whereas the differences in Cr and PCr were evident in comparison to non-helpless WT rats. Individual differences in neurochemical baselines may render some of the normal rats more vulnerable to the effects of uncontrollable stress, which could lead to the disruption of the biological system if met with further insults.

**Use of relative metabolite concentrations**

A limitation of the study was the use of relative instead of absolute metabolite concentrations. Ratios can change dependent on the numerator, the denominator or both. Although the groups did not differ with regard to tNAA/tCr or tCr/tNAA in the brain regions examined, the group differences for Glu in LHipp and RHipp were found only when tNAA was used as the denominator. When tCr was used, we found a group difference for RHipp only. In the case of Tau and PCr, cLH differed from WT rats in all three brain regions only when tCr was used as the denominator, although there were group differences for LHipp when tNAA was used in the ratio. The group differences in tCho were the most robust.

**Conclusion**

Overall, the results point to metabolic abnormalities in cLH rats even without exposure to explicit stress. The reduction in Glu and up-regulation of Tau and PCr might reflect compensatory efforts of the brain to reduce excitotoxic potential and to increase neuroprotection and energy, possibly as a result of cellular stress and damage. The reduction in tCho might represent a source or correlate of such stress. Interestingly, the correlation between metabolite level and helplessness was reversed in WT rats and might reflect an early stage of a disease process or vulnerability, whereas helplessness in cLH rats might represent a more severe form or chronic state of disease. By inference, treatments with opposite effects might be effective in the respective groups.

**Note**

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

**Acknowledgements**

The research was carried out at Brookhaven National Laboratory and was supported by Brookhaven National Laboratory Directed Research and Development Program, funded by the US Department of Energy (LDRD-07-096 to FAH), and by NYSTAR.

**Statement of Interest**

Dr Henn is a consultant for Astra Zeneca.

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