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Alteration of glial-neuronal metabolic interactions in a mouse model of Alexander disease

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Abstract

Alexander disease is a rare and usually fatal neurological disorder characterized by the abundant presence of protein aggregates in astrocytes. Most cases result from dominant missense de novo mutations in the gene encoding glial fibrillary acidic protein (GFAP), but how these mutations lead to aggregate formation and compromise function is not known. A transgenic mouse line (Tg73.7) over-expressing human GFAP produces astrocytic aggregates indistinguishable from those seen in the human disease, making them a model of this disorder. To investigate possible metabolic changes associated with Alexander disease Tg73.7 mice and controls were injected simultaneously with [1-¹³C]glucose to analyze neuronal metabolism and [1,2-¹³C]acetate to monitor astrocytic metabolism. Brain extracts were analyzed by ¹H magnetic resonance spectroscopy (MRS) to quantify amounts of several key metabolites, and by ¹³C MRS to analyze amino acid neurotransmitter metabolism. In the cerebral cortex, reduced utilization of [1,2-¹³C]acetate was observed for synthesis of glutamine, glutamate, and GABA, and the concentration of the marker for neuronal mitochondrial metabolism, N-acetylaspartate (NAA), was decreased. This indicates impaired astrocytic and neuronal metabolism and decreased transfer of glutamine from astrocytes to neurons compared to control mice. In the cerebellum, glutamine and GABA content and labeling from [1-¹³C]glucose were increased. Evidence for brain edema was found in the increased amount of water and of the osmoregulators *myo*-inositol and taurine. It can be concluded that astrocyte – neuronal interactions were altered differently in distinct regions.

Keywords

glutamate; GABA; astrocytes; neurons; ¹³C Magnetic Resonance Spectroscopy

Introduction

Mice engineered to constitutively over-express wild type human GFAP (hGFAP) form astrocyte-specific intracellular eosinophilic protein aggregates that appear identical to the Rosenthal fibers first described by Werner Rosenthal (Wippold *et al.* 2006, Rosenthal 1898, Messing *et al.* 1998). In human neuropathology, these cytoplasmic inclusions are found sporadically in association with a variety of conditions, including pilocytic astrocytomas,

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syringomyelia, and chronic gliosis. Alexander described their unusual abundance in a case of infantile progressive leukodystrophy (Alexander 1949). Subsequent discoveries of other cases with this same histopathological hallmark led to the disorder being named Alexander disease in his honor (Friede 1964). Symptoms often include delayed or arrested physical and mental development, enlargement of the head (macrocephaly) sometimes accompanied by hydrocephalus, seizures, spasticity and ataxia (reviewed in Brenner et al. 2009). The diagnosis of Alexander disease could only be confirmed by autopsy until it was discovered that most instances of the disorder were due to gain of function mutations in the GFAP gene (Brenner *et al.* 2001).

Since GFAP is primarily expressed in astrocytes in the CNS, and the Rosenthal fibers are present exclusively in these cells, it is presumed that Alexander disease results from a primary defect in astrocytes (Brenner et al. 2009). It is presently not known what functions of astrocytes are aberrant in this disease. In the present study we used ^1H and ^{13}C MRS to probe for metabolic differences in the cortex and cerebellum of hGFAP over-expressing mice. Most of the glutamate in the brain is located in glutamatergic neurons (Storm-Mathisen *et al.* 1983, Ottersen *et al.* 1992). However, most de novo synthesis of glutamate, which also is the precursor for GABA, occurs in astrocytes (ref). Glutamate is converted to glutamine in astrocytes, which is released to neurons for the synthesis of glutamate and GABA. Synaptically released glutamate is transported into astrocytes (Danbolt 2001). Thereafter, some glutamate enters the astrocytic TCA cycle via α -ketoglutarate and some is converted to glutamine directly. Glutamine is then released back to neurons and used to re-synthesize glutamate which can be stored in vesicles or enter the TCA cycle via α -ketoglutarate. In this way, astrocytes and neurons interact in glutamate metabolism through the “glutamate-glutamine cycle” (Schousboe *et al.* 1997, Danbolt 2001). The functioning of this cycle can be probed by combined injection of $[1\text{-}^{13}\text{C}]\text{glucose}$ and $[1,2\text{-}^{13}\text{C}]\text{acetate}$ and magnetic resonance spectroscopy (MRS). Acetate is selectively taken up by astrocytes since they contain a specialized transport system which is absent or less active in neurons (Waniewski & Martin 1998), whereas acetyl CoA derived from glucose has been calculated to be metabolized predominantly (70%) in the neuronal tricarboxylic acid (TCA) cycle (Hassel *et al.* 1995, Qu *et al.* 2000). Thus, by analysis of the labeling patterns of glutamate, glutamine, and GABA from glucose and acetate it is possible to draw conclusions about the contributions of astrocytes and neurons to their metabolism. Since acetate, which has a low endogenous concentration, is only metabolized by astrocytes, the rates of conversion of $[1,2\text{-}^{13}\text{C}]\text{acetate}$ into $[4,5\text{-}^{13}\text{C}]\text{glutamine}$, $[4,5\text{-}^{13}\text{C}]\text{glutamate}$ and $[1,2\text{-}^{13}\text{C}]\text{GABA}$ report on the contributions of astrocytes. Analogously, since most $[1\text{-}^{13}\text{C}]\text{glucose}$ is metabolized in neurons, the rates of incorporation of ^{13}C label from $[1\text{-}^{13}\text{C}]\text{glucose}$ into $[4\text{-}^{13}\text{C}]\text{glutamate}$, $[4\text{-}^{13}\text{C}]\text{glutamine}$ and $[2\text{-}^{13}\text{C}]\text{GABA}$ reports mostly on the contributions of neurons.

We found reduced utilization of $[1,2\text{-}^{13}\text{C}]\text{acetate}$ for synthesis of glutamine, glutamate, and GABA in the cerebral cortex. In contrast, glutamine and GABA content and labeling from $[1\text{-}^{13}\text{C}]\text{glucose}$ were increased in cerebellum. The amounts of water and the osmoregulators *myo*-inositol and taurine were increased.

Materials and Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison. Production of the mice over-expressing hGFAP has been previously described (Messing *et al.* 1998). Prior to experiments the animals had free access to food and water and were kept four per cage at a light/dark cycle of 14/10 h, humidity ~50%, temperature 22°C. Eight- to ten-week-old male transgenic mice (Tg73.7) and non-transgenic age and sex-matched controls were fasted overnight followed by IP injection of 0.3M $[1\text{-}^{13}\text{C}]\text{glucose}$ (543mg/kg) plus 0.6M $[1,2\text{-}^{13}\text{C}]\text{acetate}$ (504mg/kg). The

mice were sacrificed by decapitation 15 min later, and the head snap frozen in liquid nitrogen and stored at -80°C . Brains were removed while still frozen (cerebral cortex from 10 control and 11 Tg73.7 mice, and cerebellum from 5 control and 5 Tg73.7 mice were used). Thereafter brain tissue was homogenized in 7% (w/v) perchloric acid with a Pro200 homogenizer, and centrifuged at 4,000g for 5 min at 4°C . The pellet was re-suspended in dH_2O , centrifuged again, and the supernatants pooled and neutralized with 1 M KOH followed by lyophilization.

^{13}C MRS

A Bruker DRX-500 spectrometer (Fälladen, Germany) was used to obtain proton decoupled ^{13}C MR spectra. The samples were re-dissolved in 200 μL D_2O containing ethylene glycol 0.1% as an internal standard. Scans (typically 20,000) were accumulated with a 30° pulse angle and 30 kHz spectral width with 64K data points. The acquisition time was 1.08 s and the relaxation delay 0.5 s.

^1H MRS

The same spectrometer was used to obtain ^1H MR spectra with a sweep width of 12 kHz with 32K data points. Scans (typically 64, cortex; 520, cerebellum) were obtained with a pulse angle of 90° , an acquisition time of 1.36 s, and a relaxation delay of 10 s. Signal suppression was set at the residual water resonance.

Immunofluorescent staining

Transgenic and wild-type mice (males, 8 weeks old) were anesthetized and transcardially perfused with 4% paraformaldehyde. Brains were removed, post-fixed for 16 hrs, cryoprotected in 30% sucrose, and 40 μm sections cut with a sliding microtome. Tissues were blocked for non-specific binding and permeabilized with 5% normal donkey serum, 1% BSA and 0.25% Triton-X-100 in phosphate buffered saline for 2 hours at room temperature, followed by incubation with mouse anti-GFAP antibody (Millipore MAB3402; 1:500 in block) at 4°C overnight. After rinsing with PBS, sections were incubated with AlexaFluor-594 conjugated donkey anti-mouse secondary antibody (Invitrogen; 1:500) for 2 hours at room temperature before rinsing and mounting with VectaShield mounting media with DAPI: 4',6-diamidino-2-phenylindole (Vector Labs). Images were taken with a Diagnostic Instruments SPOT camera with equivalent exposure and gain settings for wild-type and transgenic mice within the specified brain regions.

Measurement of brain water

Brain water content was determined by the wet/dry weight method. Animals were deeply anesthetized with avertin and then sacrificed using decapitation, and the brains of 6 Tg73.7 and 4 control mice (all males and age 8 weeks) were removed from the skull. Olfactory bulb was removed and the wet weights of tissue were determined; tissue was dried for two days in an oven at 70°C ; and dry weights determined. The difference in wet/dry weights was expressed as percent water content.

Data analysis

The amounts of ^{13}C and ^1H in the different metabolites were quantified from integrals of the relevant peaks with ethylene glycol as an internal quantification standard. Due to unforeseen problems, the C-2 region was not quantifiable. In ^1H MR spectra, corrections for number of protons and ^{13}C labeled isotopomers were performed. Due to partial degradation of N-acetyl aspartate (NAA) into acetate and aspartate, it was quantified by adding the value of acetate at 1.9 ppm to that of NAA and 2 ppm. The signal to noise ratio of the $[1,2-^{13}\text{C}]\text{GABA}$ peaks

in the ^{13}C MR spectra from cerebellar samples was not adequate for quantification. All ^{13}C MR spectra were corrected for natural abundance, nuclear Overhauser and relaxation effects.

From the total amounts (Table 1) and the amounts of the ^{13}C labeled isotopomers (Table 2) it is possible to calculate the turnover for glutamate, glutamine and GABA from $[1-^{13}\text{C}]\text{glucose}$ and $[1,2-^{13}\text{C}]\text{acetate}$.

All results are given as mean $\pm$ standard deviation. Statistics were performed using the unpaired two-tailed Student's *t*-test and $p < 0.05$ was regarded as significant. Due to incomplete injection of the ^{13}C labeled compounds, two cerebral cortex samples and one cerebellum sample in the control groups could only be used for ^1H spectroscopy.

Behavioral testing

A separate cohort of age and sex-matched mice (8-week old males) were tested for motor deficits using the grip strength meter (Columbus Instruments) and accelerating rotarod (Med Associates). For grip strength, each mouse received three trials of forepaw strength. The maximum value achieved in the three trials was used for data analysis. Comparison between groups was made using an Student's *t*-test. For the rotarod, each mouse received 4 trials on one testing day, with 20–30 minutes between each trial. Each trial measured the length of time a mouse could keep walking on the rotarod during acceleration from 4–40 (rpm) over a 5 minute period, in increments of 0.12 rpm per second. Comparison between groups was made using repeated measures ANOVA.

Results

Immunofluorescent staining (Figure 2) shows dramatically elevated GFAP (red) in cortical subpial (A, B) and cerebellar (C, D) astrocytes in GFAP transgenic mice (B, D) compared with wild-type animals (A, C). DAPI stained nuclei are shown in blue.

Analysis of ^1H MR spectra from cerebral cortex and cerebellum, two discrete sites with demonstrably elevated levels of GFAP in astrocytes (see Figure 2), made it possible to quantify many metabolites. In Table 1 we list the amino acids essential for glutamatergic and GABAergic neurotransmission: glutamate, glutamine and GABA; the glycolysis related compounds lactate and alanine; the TCA cycle intermediate succinate and the neuronal marker N-acetyl aspartate (NAA); the osmolites taurine and *myo*-inositol (inositol). Compared to controls, a significant increase was detected in the concentrations of the osmolytes inositol and taurine in both brain areas of GFAP over-expressing mice (Table 1). There was a decrease in NAA in the cerebral cortex and there were significant increases in the glutamine and GABA levels in the cerebellum. No differences were detected for glutamate, lactate, alanine and succinate.

Table 2 lists the amounts of metabolites labeled by $[1-^{13}\text{C}]\text{glucose}$ and $[1,2-^{13}\text{C}]\text{acetate}$. In contrast to the data in Table 1, those in Table 2 give a dynamic picture of the mitochondrial metabolism in astrocytes and neurons that occurred during the period after injection of the labeled precursors until the termination of the experiment. In cortex there was a reduction in the amount of $[3-^{13}\text{C}]\text{glutamate}$ (Table 2). Since production of $[3-^{13}\text{C}]\text{glutamate}$ from $[1-^{13}\text{C}]\text{glucose}$ requires a full turn of the TCA cycle (see legend to Figure 1), this finding indicates reduced TCA cycle activity in glutamatergic neurons. Also in the cortex, labeling from $[1,2-^{13}\text{C}]\text{acetate}$ was generally decreased, as evidenced by reduced amounts of $[4,5-^{13}\text{C}]\text{glutamate}$, $[4,5-^{13}\text{C}]\text{glutamine}$ and $[1,2-^{13}\text{C}]\text{GABA}$ (Table 2). In contrast, in the cerebellum no significant reduction in labeling from $[1,2-^{13}\text{C}]\text{acetate}$ was observed, and there was an increase in the amount of $[4-^{13}\text{C}]\text{glutamine}$, which is derived from $[1-^{13}\text{C}]\text{glucose}$.

Even though Tg73.7 mice have altered GABA levels, they do not have pronounced motor deficiencies as revealed by the SHIRPA test (results not shown). Additional testing of motor performance was conducted using the rotarod and the grip strength meter. Performance on the rotarod was unimpaired (Figure 3A), despite a significant decrease in forepaw grip strength (Figure 3B).

Consistent with the increase in taurine and inositol, the Tg73.7 mice showed a 2.51% increase in brain water (Figure 4).

Discussion

Astrocytic - neuronal interaction

In this study we have investigated whether metabolism of astrocytes or neurons is altered in a mouse model of Alexander disease (Tg73.7) in which cortex and cerebellum have increased expression of GFAP.

Cerebral cortex—In cortex, astrocytic metabolism of Tg73.7 mice was clearly impaired. Even though glutamine content was unchanged, its rate of synthesis from acetate, i.e. [4,5-¹³C]glutamine, was reduced. Unchanged content together with decreased labeling may be explained by decreased glutamine synthesis in astrocytes, coupled to a similarly decreased degradation in neurons. It follows that transport of [4,5-¹³C]glutamine from astrocytes to neurons was diminished. This decreased transport was evidenced by the reduced amounts of [4,5-¹³C]glutamate and [1,2-¹³C]GABA in the Tg73.7 mice. However, no decrease was observed in the total amounts of glutamate and GABA, suggesting reduced degradation and/or that glutamine from astrocytes is not important for maintenance of their concentrations during the 15 minute labeling interval used in this study. The reduced amount of labeled glutamate derived from the second turn of the TCA cycle ([3-¹³C]glutamate) is consistent with reduced glutamine degradation in neurons of the Tg73.7 mice. Glutamate formed from glutamine in neurons is only partially used as a neurotransmitter, with some entering the TCA cycle to be used as an energy substrate (Yudkoff *et al.* 1989). Thus, decreased energy production in the Tg73.7 mice is evidenced by both decreased production of second cycle glutamate and decreased glutamine metabolism. Furthermore, the amount of the neuronal marker NAA, which is synthesized in mitochondria, was decreased, giving additional evidence for impaired neuronal mitochondrial metabolism in the cerebral cortex.

Metabolism in GABAergic neurons was changed differently from that of glutamatergic ones. Even though it appears likely that less glutamine was transferred to GABAergic neurons, due to the decrease in [1,2-¹³C]GABA concentration, GABA content derived from the second turn of the TCA cycle ([3-¹³C]GABA) was unchanged in the Tg73.7 mice. This is in contrast to our result for glutamatergic neurons and in support of the claim that maintenance of GABA levels is less dependent on glutamine supply, due to the fact that the re-uptake of GABA into the presynaptic neurons is greater than that into astrocytes (Schousboe 2003). The strongly impaired mitochondrial functioning of the prefrontal cortex could be part of the reason for the dementia observed in Alexander patients (Nobuhara *et al.*, 2004).

Cerebellum—There was no indication of glutamatergic hypometabolism in the cerebellum, in contrast to what was observed in the cerebral cortex. Even though glutamine metabolism was altered in astrocytes in the cerebellum of Tg73.7 mice, as evidenced by increased glutamine concentration and labeling from [1-¹³C]glucose, that of glutamate was unchanged. Normal metabolism in glutamatergic neurons could be explained by the fact that astrocyte – neuronal interactions in the cerebellar granular layer are thought to be of less

importance compared to those in other brain regions (Olstad *et al.* 2007) due to a low astrocyte to neuron ratio (Korbo *et al.* 1993, Ghandour *et al.* 1980).

We found an increase in glutamine content and labeling from [1-¹³C]glucose, i.e. [4-¹³C]glutamine in the cerebellum. Whereas in cortex, approximately 60% of glutamine is labeled from [1-¹³C]glucose in astrocytes and the remaining [4-¹³C]glutamine is derived from [4-¹³C]glutamate that is labeled in the neurons, released to the synapse, and taken up by astrocytes (Hassel *et al.*, 1997). However, as pointed out above, in the cerebellum interactions between astrocytes and glutamatergic neurons are relatively limited. Furthermore, the amount of [4-¹³C]glutamate was normal in the Tg73.7 mice. Instead, the increased amount and labeling of glutamine suggests that astrocytes in the cerebellum of the Tg73.7 mice produce more glutamine.

Metabolic markers

Our analyses demonstrated a significant increase in inositol and taurine in both brain areas investigated in the Tg73.7 mice and in brain water. All three, and glutamine which is increased in the cerebellum of the Tg73.7 mice, can be indicators of cerebral edema (Pascual *et al.*, 2007; Kakulavarapu *et al.*, 2010). The 2.51% increase in water content in the brain of the transgenic mice fits well with the increase seen in acute liver failure and ischemia (Kakulavarapu *et al.*, 2010; Gohara *et al.*, 2010). Inositol and taurine have been shown to be good indicators of edema in a head trauma model (Pascual *et al.*, 2007; Gohara *et al.*, 2010). However, in all these models a sudden intervention is responsible for the increases in water, osmolites and GFAP, whereas the Tg73.7 mice are born with a different genetic profile and it can thus be assumed that the changes observed occur gradually and not as the consequence of an acute insult. It is also formally possible that the increased water content in Tg73.7 mice reflects a difference in vascularity, and hence blood volume, rather than edema.

Inositol and taurine have also been connected to neurodegeneration. Inositol content is of particular interest, because it has been reported to be elevated in several Alexander disease cases (Dinopoulos *et al.* 2006, Sakakibara *et al.* 2007, Farina *et al.* 2008). Increase of inositol concentration is a common finding in models of neurodegeneration (Fisher *et al.*, 2002). The present results indicate that stress attributed to the overexpression of GFAP in Tg73.7 mice induced a similar inositol response as seen in other disease models. Since Tg73.7 mice have modified astrocytes, the present data support the conclusion that inositol content can be regulated by astrocytes. This finding also supports results from other authors indicating that inositol is located in astrocytes (Glanville *et al.* 1989, Fisher *et al.* 2002, Brand *et al.* 1993).

A significant increase in the levels of the osmoregulator and inhibitory neuromodulator taurine was found in both areas. Several authors have reported an increase in taurine content in studies of *in vivo* models of neuronal damage (Saransaari & Oja 2000). The occurrence of similar responses consequent to GFAP overexpression indicates that astrocyte dysfunction is sufficient to induce increased levels of taurine. In this context it is interesting that impaired release of taurine was reported from GFAP-vimentin double-knockout astrocytes after hypotonic stress (Ding *et al.* 1998).

The alterations of mitochondrial metabolism of [1-¹³C]glucose and [1,2-¹³C]acetate presented in this study were very different in cerebral cortex and cerebellum. However, increases in inositol and taurine were detected in both brain areas. It can be hypothesized that overexpression of GFAP leads to a volume increase in astrocytes necessitating a change in osmolyte concentration and the increase in water content strengthens this hypothesis

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Figure 1.

Schematic presentation of labeling in glutamate, glutamine and GABA from [1-¹³C]glucose and [1,2-¹³C]acetate. Most of the [4-¹³C]glutamate observed is synthesized from [1-¹³C]glucose and located in glutamatergic neurons; it can also be generated from [4-¹³C]glutamine from astrocytes (A). Glutamatergic neurons release [4-¹³C]glutamate which can be converted to [4-¹³C]glutamine in astrocytes, which also can synthesize [4-¹³C]glutamate via their own TCA cycle (B). [1,2-¹³C]acetate is metabolized only in astrocytes and [4,5-¹³C]glutamine can be formed (C) which may be converted to [4,5-¹³C]glutamate in glutamatergic and GABAergic neurons (C). The latter can convert this glutamate to [1,2-¹³C]GABA (C). In GABAergic neurons [2-¹³C]GABA is formed from [1-¹³C]glucose (D). Interpretation of the results obtained from ¹³C MRS is based on the different metabolic fates of [1-¹³C]glucose and [1,2-¹³C]acetate (Figure 1). [1-¹³C]glucose enters both astrocytes and neurons approximately equally (Nehlig & Coles 2007) and is transformed via glycolysis to [3-¹³C]pyruvate (Figure 1). The latter can be transported into mitochondria to enter the TCA cycle as acetyl CoA or be converted to lactate, mostly in astrocytes, to be transported to neurons (Pellerin *et al.* 2007, Bak *et al.* 2007). In neurons lactate can be oxidized to pyruvate and then be converted to [2-¹³C]acetyl CoA and enter the TCA cycle. Approximately 70% of the acetyl CoA from glucose used for glutamate synthesis in the brain is metabolized in neurons and 30% in astrocytes (Hassel *et al.* 1995, Qu *et al.* 2000). After several steps, [4-¹³C]glutamate is formed (Figure 1 A,B,D). In principal, some neuronal [4-¹³C]glutamate could be derived from [4-¹³C]glutamine synthesized in astrocytes from [1-¹³C]glucose (1B). However, Hassel *et al.* (1997) showed that [4-¹³C]glutamate formation was not impaired when astrocytic TCA cycle activity was stopped. In GABAergic neurons, [2-¹³C]GABA can be formed from [1-¹³C]glucose (Figure 1D). In astrocytes, [4-¹³C]glutamine can be synthesized from [1-¹³C]glucose (Figure 1B), but as just noted, this is apparently not a significant precursor of neuronal glutamate. In contrast, [4-¹³C]glutamate that is taken up by astrocytes, following release from neurons, can be used to synthesize [4-¹³C]glutamine (Figure 1B), and approximately 40% of [4-¹³C]glutamine is derived via this pathway (Hassel *et al.* 1997). Astrocytes can also convert [1,2-¹³C]acetate directly to [1,2-¹³C]acetyl CoA, and after several steps, produce [4,5-¹³C]glutamate and [4,5-¹³C]glutamine (Figure 1C). The [4,5-¹³C]glutamine is released and can be converted to [4,5-¹³C]glutamate in neurons. In GABAergic neurons this glutamate can be decarboxylated to [1,2-¹³C]GABA (Figure 1C). For simplicity only first turn isotopomers are shown in Figure 1. If α-[4-¹³C]ketoglutarate stays in the TCA cycle,

[3-¹³C]/[2-¹³C]glutamate can be formed. For details on the isotopomers derived from the second turn see (Melo *et al.* 2006).

Figure 2.

Increased GFAP expression in GFAP transgenic mice. Immunofluorescent staining shows dramatically elevated GFAP (red) in cortical subpial (A, B) and cerebellar (C, D) astrocytes in GFAP transgenic mice (B, D) compared with wild-type animals (A, C). DAPI stained nuclei are shown in blue. Images for wild-type and transgenic animals were taken with equivalent exposure and gain for the same regions. Scale bar = 50 μ m.

Figure 3.

Motor performance of GFAP transgenic mice (n=9) compared to littermate controls (n=8). A) Time spent on the rotarod increased across trials (indicating motor learning), but did not differ between transgenics and controls (Student's *t*-test, $p = 0.52$). B) Transgenics exhibited significantly less grip strength of forepaws compared to controls (***, $p < .001$ by Student's *t*-test). Error bars indicate ± 1 standard error of the mean.

Figure 4. Brain water in transgenic and control mice. Values in each experimental group represent percent brain water content of whole brain minus olfactory bulb. The results are expressed as mean $\pm$ SD and were analyzed with Student's *t*-test. * $p < 0.05$.

Table 1

Amounts of metabolites in cerebral cortex and cerebellum of control and Tg73.7 mice.

Metabolite	Cerebral Cortex (mmol/kg brain tissue)		Cerebellum (mmol/kg brain tissue)	
	Control (n=10)	Tg73.7 (n=11)	Control (n=5)	Tg73.7 (n=5)
Glutamate	5.8±1.3	5.4±1.2	4.8±0.9	5.4±1.1
Glutamine	1.9±0.6	1.9±0.6	1.2±0.2	1.8±0.3*
GABA	1.7±0.4	1.9±0.3	0.6±0.2	1.1±0.4*
Lactate	6.8±1.7	6.0±1.1	4.8±1.2	5.6±1.0
Alanine	0.5±0.1	0.7±0.8	0.4±0.1	0.4±0.1
Succinate	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1
NAA	5.9±0.2	5.1±0.3*	1.8±1.0	2.2±0.4
Taurine	5.6±1.4	7.3±1.5*	3.2±0.7	5.0±1.1*
Inositol	1.9±0.4	2.5±0.5*	3.3±0.8	5.3±1.3*

Brain extracts were analyzed by ^1H MRS to quantify amounts of metabolites in control and transgenic (Tg73.7) mice. The results are expressed as mean $\pm$ SD and were analyzed with Student's t-test.

*
p < 0.05

Amounts of ¹³C labeled isotopomers of glutamate, glutamine and GABA in cerebral cortex and cerebellum of transgenic (Tg73.7) and control mice.

Table 2

¹³ C Precursor	Metabolite	Cerebral Cortex ($\mu\text{mol/kg}$ brain tissue)		Cerebellum ($\mu\text{mol/kg}$ brain tissue)	
		Control (n=8 [#])	Tg73.7 (n=11)	Control (n=4 [#])	Tg73.7 (n=5)
[1- ¹³ C]glucose	[4- ¹³ C]Glutamate	411 $\pm$ 70	359 $\pm$ 89	339 $\pm$ 85	397 $\pm$ 98
Both [#]	[3- ¹³ C]Glutamate	189 $\pm$ 22	144 $\pm$ 31 *	160 $\pm$ 36	173 $\pm$ 33
[1,2- ¹³ C]acetate	[4,5- ¹³ C]Glutamate	208 $\pm$ 27	153 $\pm$ 41 *	134 $\pm$ 35	106 $\pm$ 26
[1- ¹³ C]glucose	[4- ¹³ C]Glutamine	121 $\pm$ 32	100 $\pm$ 47	51 $\pm$ 18	89 $\pm$ 22 *
Both [#]	[3- ¹³ C]Glutamine	101 $\pm$ 27	83 $\pm$ 28	60 $\pm$ 7	64 $\pm$ 16
[1,2- ¹³ C]acetate	[4,5- ¹³ C]Glutamine	252 $\pm$ 60	180 $\pm$ 70 *	142 $\pm$ 35	147 $\pm$ 28
[1- ¹³ C]glucose	[2- ¹³ C]GABA	100 $\pm$ 19	98 $\pm$ 27	48 $\pm$ 11	79 $\pm$ 28
Both [#]	[3- ¹³ C]GABA	55 $\pm$ 8	48 $\pm$ 9	25 $\pm$ 9	34 $\pm$ 11
[1,2- ¹³ C]acetate	[1,2- ¹³ C]GABA	79 $\pm$ 6	51 $\pm$ 13 *	nd	nd

Mice were injected with [1-¹³C]glucose and [1,2-¹³C]acetate and brain extracts were analyzed by ¹³C MRS in control and transgenic mice (Tg73.7). The results are expressed as mean $\pm$ SD and were analyzed with Student's t-test.

[#] Due to incomplete injection of the ¹³C labeled compounds, two cerebral cortex samples and one cerebellum sample in the control groups could only be used for ¹H spectroscopy. nd not detectable;

Both [#][1-¹³C]glucose and [1,2-¹³C]acetate can be precursors and the metabolite is from the second turn of the TCA cycle;

* p < 0.05.