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Time dependent effects of haloperidol on glutamine and GABA homeostasis and astrocyte activity in the rat brain

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Abstract

Rationale—Schizophrenia is a severe, persistent, and fairly common mental illness. Haloperidol is widely used and is effective against the symptoms of psychosis seen in schizophrenia. Chronic oral haloperidol administration decreased the number of astrocytes in the parietal cortex of macaque monkeys (Konopaske *et al. Biol Psych*, 2008). Since astrocytes play a key role in glutamate metabolism, chronic haloperidol administration was hypothesized to modulate astrocyte metabolic function and glutamate homeostasis.

Objectives—This study investigated the effects of chronic haloperidol administration on astrocyte metabolic activity and glutamate, glutamine, and GABA homeostasis.

Methods—We used *ex vivo* ¹³C magnetic resonance spectroscopy along with high performance liquid chromatography after [1-¹³C]glucose and [1,2-¹³C]acetate administration to analyze forebrain tissue from rats administered oral haloperidol for 1 or 6 months.

Results—Administration of haloperidol for 1 month produced no changes in ¹³C labeling of glutamate, glutamine, or GABA, or in their total levels. However, a 6 month haloperidol administration increased ¹³C labeling of glutamine by [1,2-¹³C]acetate. Moreover, total GABA levels were also increased. Haloperidol administration also increased the acetate/glucose utilization ratio for glutamine in the 6 month cohort.

Conclusions—Chronic haloperidol administration in rats appears to increase forebrain GABA production along with astrocyte metabolic activity. Studies exploring these processes in subjects with schizophrenia should take into account the potential confounding effects of antipsychotic medication treatment.

Keywords

¹³C magnetic resonance spectroscopy; brain; haloperidol; high performance liquid chromatography; rat; astrocyte; neuron; glutamate; glutamine; GABA

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Introduction

Schizophrenia is a chronic and severe mental illness affecting approximately 1% of the population worldwide (Jablensky 1997). Positive symptoms (e.g. hallucinations and delusions) in schizophrenia often respond to antipsychotic medications which exert their therapeutic effects mainly by antagonizing D₂-like dopamine receptors. Haloperidol, or 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one, is a butyrophenone antipsychotic medication, which was approved by the FDA in 1967. It is widely used and has high affinity for D₂ and D₄ dopamine receptors (Bymaster et al. 1996).

Several imaging studies have reported volume reductions in multiple brain regions of patients with schizophrenia (Honea et al. 2005). Brain volume changes are seen in patients having their first episode of psychosis and in patients with chronic schizophrenia. Volume changes tend to progress over time and appear to be related to clinical severity and total antipsychotic exposure (Cahn et al. 2002; Ho et al. 2011; Mathalon et al. 2001). Treatment for up to two years with haloperidol was associated with decreased whole brain grey matter volume in subjects with first-episode psychosis. Haloperidol treatment produced the largest changes in the frontal, parietal, and temporal cortices. Interestingly, chronic olanzapine treatment produced significantly less volume reductions in patients with psychosis (Lieberman et al. 2005). To explore potential mechanisms by which chronic antipsychotic treatment might produce brain volume changes in patients with schizophrenia, antipsychotic medications were administered chronically to non-human primates. In adult, male, macaque monkeys, chronic administration of oral haloperidol or olanzapine, at clinically relevant doses, produced significant reductions in brain weight and volume (Dorph-Petersen et al. 2005). The antipsychotics did not affect all brain regions equally; frontal and parietal cortices demonstrated the greatest reductions in volume. A follow-up study in the same animals revealed that haloperidol and olanzapine produced significant reductions in astrocyte numbers in the parietal cortex (Konopaske et al. 2008).

In the CNS, astrocytes provide critical metabolic support to neurons and play a key role in glutamate metabolism. After release from the presynaptic terminal, high-affinity excitatory amino acid transporters (EAATs) on the cellular membrane of astrocytes remove glutamate from the synaptic cleft (Schousboe et al. 2004). Within astrocytes, glutamine synthetase converts a portion of glutamate to glutamine, and a significant amount of glutamate is oxidatively metabolized (Hertz and Zielke 2004). Glutamine is transported back to the presynaptic terminals and phosphate-activated glutaminase (PAG) converts glutamine to glutamate, thereby replenishing the neuronal glutamate pool and tricarboxylic acid (TCA) cycle (McKenna 2007; Norenberg and Martinez-Hernandez 1979). Glutamate production depletes the neuronal TCA cycle because neurons lack an anaplerotic pathway. Thus, neurons are critically dependent on metabolic support from astrocytes, which express the anaplerotic enzyme pyruvate carboxylase (Hertz et al. 2007; Hertz and Zielke 2004).

¹³C-magnetic resonance spectroscopy (MRS) provides a powerful method to assess glutamate, glutamine, and GABA homeostasis along with neuron and astrocyte function in the brain. ¹³C is a stable isotope of carbon which has a natural abundance of 1.1%. When ¹³C-labeled glucose is injected or infused systemically, the ¹³C label becomes incorporated into glutamate, glutamine, and GABA in the brain. Acetate is preferentially metabolized by astrocytes due to differences in the transport properties of astrocytes and neurons. Astrocytes transport a much larger amount of acetate than neurons, which might indicate different expression levels of monocarboxylate transporter subtypes by the two cell types (Waniewski and Martin 1998; 2004). Therefore, using ¹³C-labeled acetate with ¹³C-labeled glucose allows simultaneous analysis of astrocytic and neuronal contributions to glutamate, glutamine, and GABA metabolism (Sonnemald and Kondziella 2003). Since

astrocytes are essential to glutamate homeostasis and chronic antipsychotic medication administration reduced astrocyte numbers in the cortex (Konopaske et al. 2008), we hypothesized that chronic haloperidol administration would alter astrocyte metabolic activity and glutamate homeostasis in the rat brain. To evaluate this hypothesis, this study used ^{13}C MRS and high performance liquid chromatography (HPLC) to assess the effects of 1 and 6 month administrations of oral haloperidol on astrocyte activity and glutamate, glutamine, and GABA homeostasis in the rat forebrain.

Materials and Methods

Animals

Twenty-six male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA) and were housed in groups of 2–4 per cage. They had a 12 hour light-dark cycle and free access to food and water. Animal housing conditions were maintained in accordance with the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources. All experimental protocols were approved by the McLean Hospital Institutional Animal Care and Use Committee. The health of the rats was evaluated daily. Rats were randomly divided into groups and received either haloperidol (1.5 mg/kg/day) or no drug in their drinking water for 1 or 6 months. Group sizes in the 1 month cohort were $n=8$ in the haloperidol group and $n=6$ in the control group. In the 6 month cohort, groups sizes were $n=6$ in both groups. To ensure adequate space in their home cages, the rats in the 6 month cohort were switched to 1 rat per cage after 5 months.

Drug Administration

Haloperidol (Sigma-Aldrich, St. Louis, MO) was dissolved in minimal glacial acetic acid and the pH was adjusted to 5.5–6.0. Animals were weighed weekly, and the volume of water consumed was measured every 3 days. The concentration of haloperidol administered was adjusted to provide approximately 1.5mg/kg/day of haloperidol to each rat based on body weight and volume of water consumed. Previously in Sprague-Dawley rats, this regimen produced plasma haloperidol levels of 6.8 ± 1.1 ng/ml and neurobiologically relevant effects (Gao et al. 1997).

Tissue Preparation

At the conclusion of drug administration, 543mg/kg (0.3M) $[1-^{13}\text{C}]$ glucose and 504mg/kg (0.6M) $[1,2-^{13}\text{C}]$ acetate (Cambridge Isotope Laboratories, Andover, MA) in distilled water were injected intra-peritoneally. After 15 minutes, the rats were sacrificed using carbon dioxide and cervical dislocation. The post-mortem interval (PMI, e.g., the time from euthanasia until the brains were frozen) was recorded for each rat. Truncal blood was obtained at the time of euthanasia, and serum was separated by centrifugation ($16,000 \times g$ for 30 minutes), then stored at -80°C until analysis. The brains were removed and flash frozen in isopentane at dry ice temperatures. The forebrains were dissected on dry ice and weighed [1 month cohort: (haloperidol: 0.62 ± 0.17 g and control: 0.60 ± 0.16 g; $p>0.05$) and 6 months cohort: (haloperidol: 0.69 ± 0.11 g and control: 0.74 ± 0.06 g; $p>0.05$)]. Forebrains were pulverized using a mortar and pestle on dry ice, then homogenized in ice-cold 10% trichloroacetic acid. Known quantities of L-homocysteine (Sigma-Aldrich, St. Louis MO) and 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Sigma-Aldrich, St. Louis MO) were added as HPLC and MRS references respectively. The samples were then centrifuged at $18,000 \times g$ for 30 minutes at 4°C . The supernatant was extracted three times with water-saturated diethyl ether to remove the trichloroacetic acid, filtered through a $0.22\text{ }\mu\text{m}$ filter, and then lyophilized using a bench-top manifold freeze dryer (Millrock Technology, Kingston, NY). The sample was reconstituted in distilled water, the pH adjusted to 7.0 with

1N NaOH, and re-lyophilized. Finally, the sample was reconstituted in 1mL of deuterium oxide.

HPLC

Total forebrain concentrations of glutamate, glutamine, and GABA were measured using a Shimadzu HPLC system with fluorescence detection (Shimadzu Corporation, Kyoto, Japan). The HPLC system included a SCL-10A controller, two LC-10 AT VP pumps, a SIL-10AD auto injector, a DGU-20A5 degasser and a RF-551 fluorescence monitor. Samples were derivatized with *o*-phthalaldehyde (OPA, Alfa Aesar, Ward Hill, MA) and *N*-*tert*-butyloxycarbonyl-L-cysteine (Boc-Cys-OH, Novabiochem, EMD Biosciences, Darmstadt, Germany). Final sample volume for HPLC was 100 μ L with 10 μ L injected into the chromatograph. Separation was accomplished by applying a binary solvent gradient to an Alltima HP column (C18, 3 μ , 150mm $\times$ 4.6mm, Grace, Deerfield, IL). Mobile phase A consisted of 25mM sodium acetate buffer at pH 6.5. Mobile phase B consisted of 100% acetonitrile. The binary gradient increased mobile phase B from 10 to 20% from minute 5 to 10 and then from 20 to 40% from minute 30 to 40. The column was re-equilibrated to starting concentrations after each run.

Ex Vivo ^{13}C MRS

Proton-decoupled ^{13}C NMR spectra were obtained at the nominal carbon radio-frequency of 75.45 MHz using a Varian Mercury 300 MHz vertical bore spectrometer (Varian Inc., Palo Alto, CA). Scans were acquired with an 18.9 kHz spectral width, 32,768 data points, and a 30° pulse flip angle. The acquisition time was 1.8 sec with a relaxation delay of 0.5 sec. Data acquisition was comprised of averaging 22,000 scans for each sample, and the temperature was held to approximately 20 °C for all scans. Preprocessing of the raw ^{13}C MRS scans acquired in the form of free induction decays (FID) included DC offset correction and zero filling of the FID to 65,536 data points. Gaussian line broadening of 3 Hz was applied followed by Fourier transformation of the FID. Finally, zero and first order phase correction and baseline correction were applied. Areas under the curve of individual peaks were measured relative to the peak for DSS using MestReNova (version 6.2, Mestre Lab Research, Santiago de Compostela, Spain).

Labeling by [1- ^{13}C]glucose and [1,2- ^{13}C]acetate

Fig. 1 reflects typical ^{13}C NMR spectra for both groups at 1 and 6 months. [1- ^{13}C]glucose is converted to pyruvate by glycolysis, which in turn, can produce [3- ^{13}C]alanine and [3- ^{13}C]lactate, or enter the TCA cycle as [2- ^{13}C]acetyl-CoA. Unlabeled oxaloacetate condenses with [2- ^{13}C]acetyl-CoA, and through exchange with [4- ^{13}C] -ketoglutarate, generates [4- ^{13}C]glutamate, [4- ^{13}C]glutamine, or [2- ^{13}C]GABA (see Fig. 2). In the second turn of the TCA cycle, labeled oxaloacetate condenses with unlabeled acetyl-CoA producing [2- ^{13}C] or [3- ^{13}C]glutamate and glutamine along with [3- ^{13}C] or [4- ^{13}C]GABA.

In astrocytes, pyruvate carboxylase (PC) can carboxylate pyruvate to oxaloacetate leading to the formation of [2- ^{13}C]glutamate and glutamine or [4- ^{13}C]GABA. Also in astrocytes, [1,2- ^{13}C]acetate can be converted to [1,2- ^{13}C]acetyl-CoA allowing the generation of [4,5- ^{13}C]glutamate, [4,5- ^{13}C]glutamine, or [1,2- ^{13}C]GABA. After a second turn of the TCA cycle glutamate and glutamine get labeled at [1,2- ^{13}C] or [3- ^{13}C], and GABA is labeled at [3- ^{13}C] or [4- ^{13}C] (Cerdan et al. 1990; Sonnewald and Kondziella 2003).

Metabolic Ratios

The calculation of acetate versus glucose utilization ratios provides information regarding the neuronal and astrocytic contributions to glutamate, glutamine and GABA production

(Kondziella et al. 2006). Acetate versus glucose utilization ratios are calculated as follows: $[4,5-^{13}\text{C}]\text{glutamate}(\text{glutamine})/[4-^{13}\text{C}]\text{glutamate}(\text{glutamine})$ and $[1,2-^{13}\text{C}]\text{GABA}/[2-^{13}\text{C}]\text{GABA}$. The ^{13}C cycling ratio reflects the length of time that the ^{13}C label stays in the TCA cycle before incorporation into glutamate and glutamine. The cycling ratio for the label from $[1-^{13}\text{C}]\text{glucose}$ is calculated as follows: $([3-^{13}\text{C}]\text{glutamate}(\text{glutamine}) - [1,2-^{13}\text{C}]\text{glutamate}(\text{glutamine}))/[4-^{13}\text{C}]\text{glutamate}(\text{glutamine})$ and $[3-^{13}\text{C}]\text{GABA}/([2-^{13}\text{C}]\text{GABA} + [1,2-^{13}\text{C}]\text{GABA})$. The cycling ratio for the label from $[1,2-^{13}\text{C}]\text{acetate}$ is calculated as follows: $[1,2-^{13}\text{C}]\text{glutamate}(\text{glutamine})/[4,5-^{13}\text{C}]\text{glutamate}(\text{glutamine})$ (Kondziella et al. 2006).

The ratio of pyruvate carboxylase to pyruvate dehydrogenase activity (PC/PDH) reflects the relative contributions of the anaplerotic and oxidative pathways to the formation of glutamate and glutamine. The PC/PDH ratio is calculated as follows:

$$([2-^{13}\text{C}]\text{glutamate}(\text{glutamine}) - ([3-^{13}\text{C}]\text{glutamate}(\text{glutamine}) + [1,2-^{13}\text{C}]\text{glutamate}(\text{glutamine}))/[4-^{13}\text{C}]\text{glutamate}(\text{glutamine}) \text{ (Eloqayli et al. 2003)}.$$

Assessment of Serum Samples

Analysis of serum samples for percent enrichment of ^{13}C in glucose and acetate was performed using mass spectrometry (Metabolic Solutions, Nashua, NH) to control for variability in the absorption of ^{13}C -labeled metabolites. If rats had <5% enrichment of either serum glucose or acetate they were not included in the MRS analyses, but they were included in the HPLC analyses.

Statistical Analyses

Analysis of covariance (ANCOVA), with group as a factor and PMI as a covariate, was used to analyze HPLC and ^{13}C MRS data. All other data were assessed using unpaired, two-tailed, *t* tests, assuming unequal variances. Results were corrected for multiple comparisons using the false discovery rate approach (Storey 2002). ANCOVAs and *t* tests were performed using the STATA statistical package (v 12.0, StataCorp, College Station, TX), and false discovery rate calculations were performed using QVALUE (v. 1.0).

Results

Initial body weights did not differ between groups ($p>0.05$), and final body weights ($\pm$ S.D.) did not differ between groups in the 1 month cohort (haloperidol: 406.1 ± 29.0 g and control: 439.3 ± 41.8 g, $p>0.05$). However, in the 6 month cohort, terminal body weights were lower in the haloperidol group (haloperidol: 614.2 ± 42.9 g and control: 769.7 ± 63.4 g, $p<0.05$). Average PMI ($\pm$ S.D.) did not differ between groups at either time point ($p>0.05$; 1 month: haloperidol: 290.9 ± 107.4 sec. and control: 258.5 ± 184.2 sec.; 6 month: haloperidol: 376.3 ± 99.7 sec. and control: 379.5 ± 62.8 sec.). In both the 1 and 6 month cohorts, percent enrichment ($\pm$ S.D.) of ^{13}C in serum glucose (1 month: haloperidol: $21.5\pm3.3\%$ and control: $23.8\pm7.7\%$; 6 month: haloperidol: $22.9\pm1.5\%$ and control: $14.4\pm6.2\%$) and in serum acetate (1 month: haloperidol: $33.6\pm3.6\%$ and control: $36.6\pm15.2\%$; 6 month: haloperidol: $48.7\pm8.6\%$ and control: $46.3\pm7.4\%$) did not differ between groups ($p>0.05$).

HPLC

There was a statistically significant correlation between PMI and total glutamate levels ($r=-0.57$, $p<0.05$) across all rats, but not with total glutamine or total GABA levels. Total glutamate, glutamine, and GABA levels were not different between groups in the 1 month cohort ($p>0.05$). However, in the 6 month cohort, total GABA was significantly increased ($p<0.05$) in the haloperidol group (see Fig. 3).

Ex Vivo ^{13}C MRS

There was no statistically significant correlation between PMI and ^{13}C -labeled metabolites across all rats. Labeling of glutamate, glutamine, and GABA by $[1-^{13}\text{C}]$ glucose- and $[1,2-^{13}\text{C}]$ acetate-derived ^{13}C did not differ between groups in the 1 month cohort. Labeling of glutamate, glutamine, and GABA by $[1-^{13}\text{C}]$ glucose-derived ^{13}C also did not differ between groups in the 6 month cohort. However, in the 6 month cohort, $[4,5-^{13}\text{C}]$ glutamine ($p < 0.05$) levels were increased significantly in the haloperidol group (see Fig. 4).

As seen in Table 1, administration of oral haloperidol increased the acetate/glucose utilization ratio for glutamine in the 6 month cohort ($p < 0.05$), but not in the 1 month cohort ($p > 0.05$). Acetate/glucose utilization ratios for glutamate and GABA in both cohorts were not different between groups ($p > 0.05$). Neither the ^{13}C cycling ratios for $[1-^{13}\text{C}]$ glucose and $[1,2-^{13}\text{C}]$ acetate or the PC/PDH ratios differed between groups in either cohort ($p > 0.05$, Data not shown).

Discussion

In male, Sprague-Dawley rats, oral administration of haloperidol for 6 months, but not 1 month, increased total GABA levels. The labeling of glutamine by $[1,2-^{13}\text{C}]$ acetate and the acetate/glucose utilization ratio for glutamine were increased at 6 months in the haloperidol group, but were unchanged in the 1 month cohort. Together, these data suggests that haloperidol administration increased GABA production and astrocytic metabolic activity after 6 months, but not after 1 month. The following discussion will explore the mechanisms by which haloperidol might have produced these effects.

Previous studies have examined the effects of chronic haloperidol on GABA levels in subcortical and cortical regions. A 6 month haloperidol administration in rats increased GABA release in the globus pallidus, but not in the striatum (Grimm and See 2000; See and Chapman 1994), whereas a briefer 28 day administration increased GABA release in the rat striatum (Osborne et al. 1994). In contrast to the current study, which examined the entire forebrain (cortex and striatum), administration of haloperidol for 6 months in rats did not change GABA levels in smaller brain areas including medial frontal cortex and striatum (Bustillo et al. 2005; See and Lynch 1995). In addition to GABA levels, several studies examined the effects of haloperidol administration on components of the GABAergic system. In two studies, a 6 month haloperidol administration in rats increased GABA_A receptor binding in the striatum (Shirakawa and Tamminga 1994; Zink et al. 2004b), but produced conflicting results in the cortex. Zink *et al.* (2004) found increased GABA_A receptor binding in multiple cortical areas, but Shirakawa *et al.* (1994) found no change in the cortex. Interestingly, a 19 week administration increased GABA-immunoreactive labeling in axosomatic terminals on pyramidal cells in the rat prefrontal cortex (PFC) (Vincent et al. 1994).

In the current study, administration of haloperidol for 6 months increased total GABA levels suggesting that haloperidol produced a net increase in GABA production. In the brain, the rates of production, along with re-uptake and subsequent metabolism, regulate GABA levels. GABA is synthesized from glutamate in interneurons by glutamic acid decarboxylase (GAD₆₇) and is removed from the extracellular space by the GABA transporters. In rats, both 28 day and 6 month haloperidol administrations increased GAD₆₇ mRNA expression in the cortex, but only the 28 day administration increased GAD₆₇ mRNA expression in the striatum (Lipska et al. 2003; Zink et al. 2004b). A 6 month administration in rats increased the mRNA expression of a GABA transporter, GAT-1, in multiple cortical areas including frontal cortex (Zink et al. 2004a). Together, these data suggest that chronic haloperidol affects the GABAergic system in cortical and subcortical areas. Moreover, the increased

total GABA levels might reflect increased GAD₆₇ expression and therefore, increased GABA production in the frontal cortex. The 6 month haloperidol administration might have also led to increased GAT-1 expression in the frontal cortex to compensate for increased GABA production (Bernstein and Quick 1999).

Consistent with previous reports, the current study found no change in total glutamate levels after 1 and 6 months of haloperidol administration. A 21 day haloperidol administration in rats increased glutamate levels in the striatum, but not in the PFC (Yamamoto and Cooperman 1994; Yamamoto et al. 1994). However, a 6 month administration in rats produced no change in basal glutamate levels in the striatum or medial PFC (Bustillo et al. 2005; See and Chapman 1994; See and Lynch 1995).

The mechanism by which chronic haloperidol increased GABA production might be related to its receptor binding profile and its impact on neurocircuitry. Using various cellular and tissue systems derived from rats, hamsters, cows, and humans, Bymaster *et al.* (1996) found that haloperidol bound to D₂ and D₄ dopamine receptors with high affinity and had moderate affinity for D₁. It interacted with 5-HT_{2A} receptors with moderate affinity and had a very low affinity for other serotonin receptor subtypes and muscarinic receptors (Bymaster et al. 1996). Haloperidol had moderate affinity for α_1 adrenergic, but low affinity for α_2 adrenergic and H₁ histamine receptors. Notably, haloperidol did not bind to GABA_A receptors (Bymaster et al. 1996). In a similar study, conducted in human brain tissue homogenates, haloperidol had high affinity for D2 dopamine receptors, and moderate affinity for serotonin 5-HT_{2A}, and 5-HT_{1D} receptors. It had moderate affinity for α_1 adrenergic receptors and low affinity for α_2 adrenergic, H1 histaminergic, and 5-HT_{1A}, and 5-HT_{2C} serotonergic receptors. Haloperidol did not appear to bind M₁ muscarinic receptors (Richelson and Souder 2000). Together these data suggest that haloperidol's main pharmacologic mechanism of action involves antagonism of dopamine D₂ receptors.

Although haloperidol has modest affinity for 5-HT_{2A} receptors, activity at 5-HT_{2A} is unlikely to produce the increased total GABA levels observed in the present study (Abi-Saab et al. 1999; Bourdelais and Deutch 1994; Cozzi and Nichols 1996). Haloperidol's strong D₂ dopamine antagonism likely plays a prominent role in its effect on GABA production. In rats, haloperidol increased dopamine levels in both the striatum and PFC, albeit to a greater degree in the striatum (Kuroki et al. 1999; Liegeois et al. 2002). The dorsal striatum (caudate-putamen) receives dopaminergic input from the substantia nigra (nigro-striatal pathway), whereas the ventral striatum (nucleus accumbens) receives dopaminergic input from mainly the ventral tegmentum (mesolimbic pathway) (Girault 2012). Most striatal neurons are medium spiny neurons, which utilize GABA as their neurotransmitter and express dopamine D₂ receptors (Delle Donne et al. 1997; Harsing and Zigmond 1997). Stimulating the rat striatum resulted in increased GABA release, and dopamine D₁ agonists and D₂ antagonists enhanced while D₂ agonists inhibited this effect (Harsing and Zigmond 1997; Momiyama and Koga 2001; Steulet et al. 1990). Dopamine afferents seem to have a tonic inhibitory effect on GABAergic neurons in the rat striatum (Lindfors et al. 1989a). Indeed, dopamine de-afferentation of the rat striatum resulted in increased GAD₆₇ activity and GABA levels in the striatum (Lindfors et al. 1989b; Nagy et al. 1978). Although these findings would seem to indicate that chronic D₂ blockade would increase GABA levels in the striatum, several studies have found no change in GABA levels after a 6 month haloperidol administration in rats (Bustillo et al. 2005; Grimm and See 2000; See and Chapman 1994; See and Lynch 1995). Interestingly, a briefer administration of 28 days in rats did result in increased striatal GABA levels (Osborne et al. 1994), suggesting the development of tolerance with longer haloperidol administration (Hess et al. 1988). Thus, the higher GABA level observed after the 6 month haloperidol administration in the

present study might be due to increased production in areas outside the striatum, such as the frontal cortex.

Dopaminergic fibers from the ventral tegmentum (mesocortical pathway) densely innervate the PFC. GABAergic interneurons, especially those containing the calcium buffering protein parvalbumin, express dopamine D₁ and D₂ receptors in the PFC (Khan et al. 2001; Le Moine and Gaspar 1998; Muly et al. 1998). In the rat PFC, D₁ agonists tended to increase, whereas D₂ agonists tended to reduce GABAergic neurotransmission (Seamans et al. 2001). In addition, the regulation of GABA release by dopamine input in the PFC appears to be concentration-dependent. At low concentration, dopamine preferentially acts at D₁ receptors and increased GABA release, whereas at high concentrations, dopamine activated both D₁ and D₂ receptors, but the D₂-mediated reduction in GABAergic neurotransmission predominates (Trantham-Davidson et al. 2004). Chronic blockade of dopamine D₂ receptors by haloperidol in the cortex would make D₁ receptors relatively unopposed. Thus, in the current study, activity at unopposed D₁ receptors in the frontal cortex might have contributed to the increased GABA production seen after the 6 month haloperidol administration.

Although the current study did not evaluate total astrocyte numbers, chronic antipsychotic medication administration reduced total astrocyte numbers in the parietal cortex of macaque monkeys (Konopaske et al. 2008). Thus, chronic haloperidol administration might have reduced astrocyte numbers in the forebrain. Nevertheless, the increased labeling of glutamine by [1,2-¹³C]acetate coupled with the increased acetate/glucose utilization ratio for glutamine at 6 months in the haloperidol group suggests that haloperidol increased astrocyte metabolic activity. Haloperidol's modulation of dopamine neurotransmission might explain, at least in part, the increase in astrocyte metabolism observed in the current study. Indeed, astrocytes were found to express functional D₁ and D₂ receptors (Bal et al. 1994; Khan et al. 2001; Vermeulen et al. 1994), and application of dopamine to murine astrocyte cultures resulted in increased intracellular calcium signaling (Requardt et al. 2012). In astrocytes, the NAD⁺/NADH redox pair modulates intracellular calcium signaling and plays a key role in multiple metabolic pathways including the mitochondrial respiratory chain and lactate shuttle. Dopamine induced changes in the NAD⁺/NADH redox state in cultured murine astrocytes via D₁ receptor signaling (Requardt et al. 2012; Requardt et al. 2010). Thus, chronic blockade of D₂ receptors on astrocytes by haloperidol could result in activation of astrocyte metabolic activity by unopposed D₁ receptor stimulation.

In addition to its modulation of astrocyte stimulation by dopamine, haloperidol may have also increased astrocyte metabolic activity indirectly through its anorectic effect. In patients with schizophrenia, treatment with haloperidol produced minimal, if any, changes in body weight (Basson et al. 2001). Haloperidol administration significantly reduced total body weight among rats in the 6 month cohort, but not in the 1 month cohort. Prior studies have found similar effects of haloperidol on body weight in rats. 9 months of oral haloperidol in male Sprague-Dawley rats decreased body weight and spontaneous activity (Waddington and Gamble 1980), but only 6 weeks of oral haloperidol did not change oral intake or body weight (Minet-Ringuet et al. 2005). In the 6 month cohort, haloperidol might have modulated the effects of leptin leading to reduced body weight. Leptin is a 16 kDa hormone which is released by adipose cells and binds to receptors in the hypothalamus where it modulates food intake and metabolism. Dopamine innervates the hypothalamus via the mesolimbic pathway and the binding of dopamine to D₂ receptors in the hypothalamus inhibits the effects of leptin. Notably, D₂ knockout mice exhibited decreased body weight, food intake and serum leptin, but had increased energy expenditure and leptin sensitivity. Central leptin injection in wild-type mice decreased food intake and this effect was magnified by haloperidol (Kim et al. 2010). Although 6 weeks of oral haloperidol did not

alter leptin levels or hypothalamic leptin receptor expression in rats (Minet-Ringuet et al. 2005; von Wilmsdorff et al. 2010), 6 months of haloperidol administration in the current study might have modulated the activity of leptin by blocking D2 receptors in the hypothalamus, producing an anorectic effect.

In a previous study, rats fed a ketogenic diet (i.e., high fat with low protein and carbohydrates) exhibited increased astrocyte metabolic activity in the cortex. Unlike haloperidol-administered rats, the rats on a ketogenic diet also exhibited increased neuronal metabolic activity in the cortex perhaps reflecting dietary differences or that they were bred to develop absence epilepsy (Melø et al. 2006). Nevertheless, this study supports the notion that in addition to its blockade of D2 receptors on astrocytes, chronic haloperidol administration may have indirectly contributed to increased astrocyte metabolic activity by antagonizing D2 receptors in the hypothalamus producing an anorectic effect.

Haloperidol's lack of significant effects after 1 month and production of increased GABA levels and astrocyte metabolic activity after 6 months raise the question of what might underlie these time-dependent findings. In rats, the neurobiological effects of chronic D₂ antagonist administration were found to change over time (Hess et al. 1988). Indeed, in patients with schizophrenia, treatment with antipsychotic medication usually requires several weeks to produce a clinical improvement in psychotic symptoms (Kasper et al. 2003). Thus, the effects of haloperidol observed after 6 months in the current study likely reflect late-onset changes in gene transcription or translation related to chronic D₂ antagonism (Eastwood et al. 1997).

Findings from the current study have relevance to studies of patients with schizophrenia. ¹H magnetic resonance spectroscopy has been used to determine levels of brain metabolites *in vivo*, and demonstrated increased glutamine levels in the left anterior cingulate and left thalamus in subjects with first-episode schizophrenia. Furthermore, no change in glutamate levels was observed (Theberge et al. 2002). Patients with chronic schizophrenia had decreased glutamine and glutamate levels in the left anterior cingulate with increased glutamine levels in the left thalamus (Theberge et al. 2003). Moreover, patients with chronic schizophrenia also showed increased GABA levels in the anterior cingulate and parieto-occipital cortex. No change in glutamate levels was observed (Ongur et al. 2010). The results of the current study suggest that the higher GABA levels observed in subjects with chronic schizophrenia (Ongur et al. 2010) might be due to antipsychotic medication treatment. Thus, studies of schizophrenia assessing brain metabolites and astrocyte metabolic function should take into account the potential confounding effects of antipsychotic medication treatment. In addition, the significant correlation of PMI with total glutamate levels in the current study highlight the need to account for the potential confounding effects of PMI in post-mortem human studies assessing brain metabolites in schizophrenia and other neuropsychiatric disorders.

In conclusion, haloperidol, in a time-dependent fashion, increased GABA production and astrocyte metabolic activity in the forebrain. These findings are likely due, directly and indirectly, to chronic D₂ receptor antagonism by haloperidol. Since D₂ antagonists might modulate brain metabolite levels and astrocyte metabolic function, studies exploring these parameters in subjects with schizophrenia should take into account the possible effects of antipsychotic medications, especially potent D₂ antagonists like haloperidol.

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The animals experiments conducted in this study are in compliance with applicable State and Federal (United States) regulations.

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Fig. 1. Typical ^{13}C NMR spectra of control (A and C) and haloperidol-exposed (B and D) rats. The 1 month cohort is represented in the upper panels (A and B) with the 6 month cohort being represented in the lower panels (C and D). Peak assignments: 1: C3-glutamine; 2: C3-glutamate; 3: [4,5- ^{13}C]glutamine; 4: C4-glutamine; 5: [4,5- ^{13}C]glutamate; 6: C4-glutamate; 7: C2-/C3-succinate; 8: C2-GABA; 9: C3-aspartate.

Fig. 2. $[1-^{13}\text{C}]$ glucose and $[1,2-^{13}\text{C}]$ acetate labeling patterns of glutamate, glutamine, and GABA. Filled circle represents ^{13}C and open circle represents unlabeled.

Fig. 3.

Total concentrations ($\mu\text{mol/g}$) of forebrain metabolites in control (open bars) and haloperidol-administered (filled bars) rats, measured by HPLC. Panel A represents the 1 month cohort and panel B represents the 6 month cohort. Values represent mean $\pm$ S.D., * $p < 0.05$.

Fig. 4.

Amounts of forebrain metabolites (nmol/g) labeled by $[1-^{13}\text{C}]$ glucose (Panels A and C) and by $[1,2-^{13}\text{C}]$ acetate (Panels B and D) in control (open bars) and haloperidol-administered (filled bars) rats, measured by ^{13}C MRS. The 1 month cohort is represented in the upper panels (A and B) with the 6 month cohort being represented in the lower panels (C and D). Values represent mean $\pm$ S.D., * $p < 0.05$.

Table 1

Acetate/Glucose Utilization Ratios from control and haloperidol-administered rats.

	Acetate/glucose Utilization		
	Glutamate	Glutamine	GABA
<i>1 month</i>			
Haloperidol	0.19±0.02	1.38±0.14	0.15±0.1
Control	0.17±0.06	1.01±0.31	0.17±0.08
<i>6 months</i>			
Haloperidol	0.23±0.03	1.56±0.33 *	0.11±0.09
Control	0.18±0.04	0.76±0.25	0.12±0.1

Mean values ± S.D.,

* = p < 0.05