

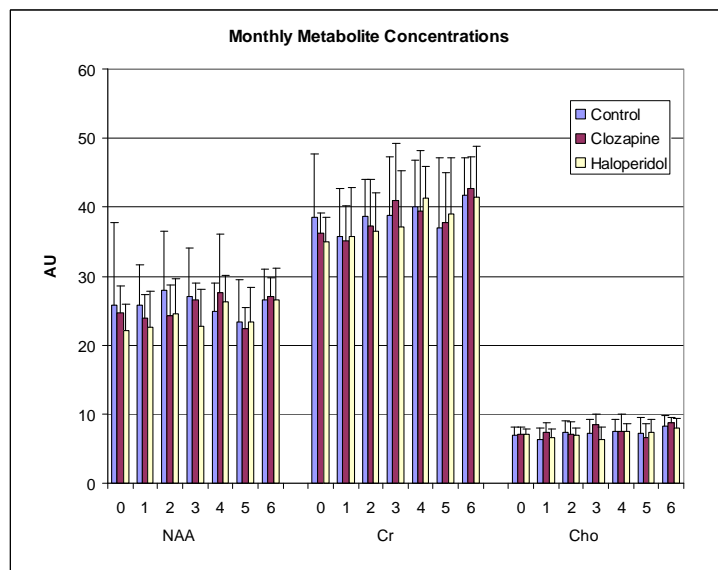
Long-term Antipsychotic Treatment Does Not Alter MRS Measures of Metabolite Levels in Normal Rat Brain

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Introduction: Schizophrenia affects approximately 1% of the world population. Multiple MRS studies have been reported comparing schizophrenic patients with normal subjects, but the results have been mixed. In general, N-acetylaspartate (NAA) levels are decreased in the frontal and temporal lobes of schizophrenic patients, although reductions of NAA have been reported throughout the brain. Since patients usually are stabilized on antipsychotics when they are examined, any change in metabolite concentrations could result from the disease and/or the drugs used to treat it. Typical antipsychotics, such as haloperidol, target primarily dopamine receptors, whereas atypical antipsychotics, such as clozapine, target multiple receptors, which might lead to differing effects on metabolite levels. In a study comparing the effects of haloperidol and clozapine, Bustillo's group found that haloperidol-treated patients had lower frontal NAA levels than clozapine-treated patients (1). Heimberg et al. reported that patients treated with typical antipsychotic medications had lower left frontal NAA/creatine (Cr) than patients treated with atypical antipsychotics (2). Ende et al. have shown greater reductions in anterior cingulate NAA in patients treated with typical antipsychotics than in those treated with atypical antipsychotics (3, 4). Recently, however, a more systematic study by Bustillo found no treatment effects on NAA levels in drug-naïve or minimally-treated schizophrenic patients (5). Differentiating the effects of disease from treatment, without confounds from diet, lifestyle, other drugs, age or other factors, is difficult in a patient population. Here we report the results of a 6-month study in normal rat brain to determine the effects of antipsychotics on metabolite levels.

Methods: The institutional animal use committee approved this study. Two groups of male Sprague-Dawley rats (n=7/group) were dosed with approximately 2 mg/kg/day haloperidol or 30 mg/kg/day clozapine via drinking water for 6 months. Control rats (n=9) were given water. Animals were anesthetized with 1.5% isoflurane in air during scans. 1H MRS data were acquired from each rat prior to and monthly during antipsychotic dosing from a 4 mm/side voxel in the left thalamus using a 7T Bruker BioSpec system. After shimming on the voxel, PRESS spectra were acquired with and without water suppression at TE 20 ms and TR of 6 s. To assess the CSF content, non-suppressed data were acquired at 12 different TEs with a TR of 2.5 s. All data were processed automatically using Bruker routines to correct for eddy currents and phase. LCModel (6) was used to estimate the metabolite concentration referenced to brain water in institutional units; results were used if the Cramer-Rao lower bounds were less than 20% for all metabolites except inositol (Ins), where a cut-off of 30% was used. Data were analyzed using SPSS 12.0 for Windows.



Results: CSF accounted for 3% or less of the water fraction in all voxels. All data were acceptable for NAA, total creatine (Cr), and total choline (Cho). For glutamine +glutamate (Glx), data from 7 control, 6 haloperidol-, and 7 clozapine-treated rats were acceptable. For Ins, data from 8 control, 5 haloperidol-, and 7 clozapine-treated rats were acceptable. Repeated measures analysis with time as the within-subjects measure and treatment as the between-subjects factor showed no treatment effect for any metabolite. There was a significant effect of time for Cr and Cho ($p < 0.003$). Monthly average values for NAA, Cr, and Cho are shown in the figure.

Discussion: These data are not corrected for relaxation times, which may independently alter metabolite concentrations. However, the short TE and long TR used for this study were chosen to minimize relaxation effects. CSF contamination was minimal throughout this study and would not, therefore, mask any relevant changes in metabolite level. Brain water content changes significantly in the first month of life and then gradually decreases; animals entered this study at approximately 6 weeks of age, after the greatest change, so we assumed no significant change in water content over the course of this study.

To our knowledge, this is the first longitudinal study comparing clozapine and haloperidol using *in vivo* measurements of multiple

metabolites in the rat. Our results are in agreement with those of Bustillo, et al. (5), who report no effect of treatment on NAA, Cr, or Cho. Although changes might be expected in Glx or Ins, since alterations in both have been implicated in schizophrenia (7, 8), we found no changes in this study.

Although there is *in vitro* evidence to suggest that these drugs do have differential effects on NAA levels in neuronal cells (9), our results suggest that these effects are minor *in vivo*. The results are in agreement with *in vitro* MAS-NMR experiments by Bustillo (10), but contradict those of Harte (11). Harte, et al., used tissue extracts and HPLC to analyze metabolite levels, which might have detected NAA that is not visible with *in vivo* MRS or MAS-NMR of tissue samples. Both this study and Bustillo's recent study (5) were longitudinal and neither found any treatment effect at any time point for NAA. Taken together, these results indicate that reductions in NAA levels seen in schizophrenic patients compared with normal subjects are the result of the disease.

In conclusion, our results indicate that antipsychotic drugs do not significantly alter the concentrations of any metabolite. Changes seen in treated schizophrenic patients, therefore, are likely due to the disease itself.

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