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Blood harmine is correlated with cerebellar metabolism in essential tremor
A pilot study

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ABSTRACT

Background: On proton magnetic resonance spectroscopic imaging (1H MRSI), there is a decrease in cerebellar N-acetylaspartate/total creatine (NAA/tCr) in essential tremor (ET), signifying cerebellar neuronal dysfunction or degeneration. Harmame, which is present in the human diet, is a potent tremor-producing neurotoxin. Blood harmame concentrations seem to be elevated in ET.

Objectives: To assess in patients with ET whether blood harmame concentration is correlated with cerebellar NAA/tCr, a neuroimaging measure of neuronal dysfunction or degeneration.

Methods: Twelve patients with ET underwent 1H MRSI. The major neuroanatomic structure of interest was the cerebellar cortex. Secondary regions were the central cerebellar white matter, cerebellar vermis, thalamus, and basal ganglia. Blood concentrations of harmame and another neurotoxin, lead, were also assessed.

Results: Mean ± SD cerebellar NAA/tCr was 1.52 ± 0.41. In a linear regression model that adjusted for age and gender, log blood harmame concentration was a predictor of cerebellar NAA/tCr (beta = −0.41, p = 0.009); every 1 g⁻¹/mL unit increase in log blood harmame concentration was associated with a 0.41 unit decrease in cerebellar NAA/tCr. The association between blood harmame concentration and brain NAA/tCr only occurred in the cerebellar cortex; it was not observed in secondary brain regions of interest. Furthermore, the association was specific to harmame and not another neurotoxin, lead.

Conclusion: This study provides additional support for the emerging link between harmame, a neurotoxin, and ET. Further studies are warranted to address whether cerebellar harmame concentrations are associated with cerebellar pathology in postmortem studies of the ET brain.

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Essential tremor (ET) is a common neurologic disorder.¹-³ Proton magnetic resonance spectroscopic imaging (1H MRSI) has revealed decreased cerebellar N-acetylaspartate/total creatine (NAA/tCr) in patients with ET,¹ which is suggestive of neuronal dysfunction or degeneration, and recent postmortem studies have demonstrated mild degenerative changes in the ET cerebellum.⁵,⁶ Genetic factors clearly play a role in disease etiology.⁷ Environmental factors likely also play a role.⁸,⁹ Harmame (1-methyl-9H-pyrido[3,4-b]indole), a potent neurotoxin,¹⁰,¹¹ is present in the human diet in numerous food items.¹²-¹⁵ As a heterocyclic amine (HCA),¹⁰ it is made up of several five- and six-ringed structures, which contain an amine group. There is a structural similarity to the neurotoxin MPTP, which has a two-ring structure. Laboratory animals acutely exposed to harmame and other HCAs develop tremor¹¹,¹⁶,¹⁷ accompanied by destruction of inferior olivary and cerebellar tissue.¹⁸ Indeed, harmame is among the most tremorogenic HCAs.¹¹ Furthermore, it is one of the most abundant of all dietary HCAs,¹⁹,²⁰ its concentrations are readily detectible in the blood,²¹ and blood harmame concentration (but not that of...
harmine, for example) was found to be elevated in ET cases. The mechanisms for this elevation, although unclear, could include increased dietary intake or genetic-photocatalytic factors. Harmane crosses the blood–brain barrier through an active uptake mechanism and, although related, brain concentrations are higher than those seen in the blood. Our goal was to further examine the emerging link between this neurotoxin and ET. In the current study, we hypothesized that blood harmane concentration in ET cases would be correlated with cerebellar NAA/tCR, a neuroimaging measure of neuronal dysfunction or degeneration.

**METHODS Study subjects.** Patients with ET were identified from a computerized database at the New York Neurological Institute and were enrolled in an epidemiologic study of ET. All patients had received a diagnosis of ET from their treating neurologist. Office records were reviewed and patients with PD, dementia, dystonia, or stroke were excluded. All met published diagnostic criteria for ET. Twenty-four patients were selected for MRSI based on proximity to the medical center; four refused. The remaining 20 potential subjects were screened over the telephone using a brief neurologic disease questionnaire and a 10-minute cognitive assessment (the Telephone Interview for Cognitive Status); patients without evidence of other neurologic disorders or cognitive impairment (score ≥31 of 41) were enrolled. Twenty patients with ET were enrolled and underwent MRSI.

**Videotaped examination and tremor ratings.** A videotaped tremor examination included one test to elicit postural tremor and five tests to elicit kinetic tremor; each test was performed with the dominant and nondominant arms. Each videotape was reviewed (E.D.L.) without knowledge of MRSI results, and the tremor was rated on each test from 0 to 3 (severe tremor). A total tremor score (0 to 36) was calculated for each patient by the addition of the 12 scored items.

**1H MRSI protocol.** The multislice 1H MRSI studies were performed on a 1.5 T GE Signa Scanner with the method described. Following 13 standard T1 sagittal brain MRI scout images, a four-section T1-weighted axial-oblique MRSI localizer image was acquired at the same slice locations using the same slice thickness as was to be used in the 1H MRSI scan. This would permit correlation and co-registration of MRI and 1H MRSI data. Multislice 1H MRSI data were acquired by prescribing four 15-mm axial-oblique slices, which were angulated such as to achieve coverage of the cerebellum and other regions of interest (ROIs). To ensure reproducible positioning of slices from patient to patient, the four MRSI slices were oblique such that the first slice traversed the plane defined by two anatomic landmarks, the superior cerebellar peduncle and the anterior commissure. This multislice 1H MRSI method employs a standard slice-interleaved spin echo sequence except that it used octagonally tailored outer volume presaturation pulses to suppress the strong lipid signals arising from the scalp, skull, and calvarial marrow. The MRSI spectral data were recorded using an echo time of 280 msec, repetition time of 2,300 msec, field of view of 240 mm, 32 × 32 phase-encoding steps with circular k-space sampling, and 256 points along the signal acquisition domain. The resulting nominal MRSI voxel size was 1.5 cm × 0.75 cm × 0.75 cm.

MRSI data were transferred to an off-line workstation for processing and analysis by two of the study investigators (X.M. and D.C.S.) who used interactive MRSI data analysis software of their own design. Data analysis was performed blind to the clinical information. These two study investigators had no visual or verbal contact with the patients and did not have access to data on their demographic characteristics, clinical features, or diagnoses. The raw data were sorted by slice, zero-filled twice along the acquisition domain (to 1,024 sampling points), filtered with a Gaussian-Lorentz window and a Hamming window along the time and spatial domains, and then processed by standard three-dimensional fast Fourier transformation to yield an array of 32 × 32 spectra. The spectral data were automatically corrected for susceptibility shifts due to slight variations in magnetic field strength across the brain.

A neuroradiologist, shielded from the clinical information, chose MRSI voxels of interest using a registered grid overlay on the axial-oblique T1-weighted MRSI localizer images. Based on published results, the major neuroanatomical structure of interest was the cerebellar cortex. Secondary ROIs included the central cerebellar white matter, cerebellar vermis, thalamus, and basal ganglia; metabolic changes in ET have not been demonstrated in any of these secondary regions in prior analyses. All ROIs included both right and left sides, except for the vermis. The cerebellar cortex was delineated by those voxels that approximated the lateral and posterior surfaces of the cerebellum on axial imaging, and the central cerebellar white matter ROI included voxels that approximated the medial/inner parenchyma of the cerebellum. For the basal ganglia, voxels in the head of the caudate and lenticular nuclei were selected.

Relative metabolite peak areas for each of these ROIs were calculated as follows. MRSI voxels were drawn to scale within these ROIs and their locations noted for coregistration with metabolite images. In each voxel, metabolite peak areas were obtained using a nonlinear least-squares curve fitting routine. As many pixels as possible were fitted within each ROI and then averaged. The number of voxels per ROI was variable. The mean (range) number of voxels per side was as follows: cerebellar cortex, 6 (4 to 7); central cerebellar white matter, 5 (4 to 7); cerebellar vermis, 2 (2 to 4); basal ganglia, 4 (3 to 5); and thalamus, 2 (2 to 3). All voxels of interest were selected manually by two experienced MR spectroscopists (D.C.S., X.M.). They used as a rejection criterion the lack of a clear separation between the total choline (tCho) and tCr resonances. This criterion was applied regardless of the status of the NAA peak. The integrated area of the NAA peak could be reliably ascertained by spectral analysis, which was fully automated in this study, thereby minimizing investigator bias. In the absence of a separation between the tCho and tCr resonances, most automated spectral fitting programs might meet the least-squares convergence criteria, but would generally not yield reliable peak areas. Therefore, voxels with spectra not meeting this rejection criterion were excluded from the analysis. X.M. had...
access to anatomic images but no access to clinical/diagnostic data so that X.M. had no knowledge of case-control status while selecting voxels. The senior spectroscopist, D.C.S., reviewed all voxels prior to final exclusion. D.C.S. was blinded to clinical/diagnostic data and anatomic images. In each subject, between one and four voxels in total were excluded because of poor spectral quality (unresolved resonances). However, this did not result in the exclusion of any patients. Pixels corresponding to the ROI on the metabolite images were extracted, and their sample means computed pixel by pixel for use in statistical tests. The major metabolite detected was NAA, expressed as a ratio to tCR.

Blood harmane concentration. The determination of blood harmane concentration was later added to the study protocol when funding became available. Hence, phlebotomy was performed on 12 of the 20 ET cases who had had MRSI. These 12 were compared to the 8 ET cases who did not undergo phlebotomy and there were no differences in age, gender, race, total tremor score, or cerebellar NAA/tCR.

Evaluations were performed in the late morning or early afternoon (between 10 and 2 PM), making fasting levels impractical. Data suggest that plasma concentrations of harmane do not change significantly after meals. In one study, human subjects ingested food or ethanol, and plasma harmane concentrations were measured hourly for 8 hours. The concentration remained stable. The same investigators also demonstrated that variability in concentration was minimal over a longer (3-week) period.

Blood lead concentration. Blood concentrations of harmane were measured blinded to all clinical and MRSI information. A novel high performance liquid chromatography (HPLC) method for quantifying harmane in blood has been reported. Briefly, one volume (9 to 12 mL) of whole blood was digested with NaOH, extracted with ethyl acetate and methyl-t-butyl ether (2:98, V:V), and reconstructed in methanol. Harmane was separated and quantified by HPLC with a fluorescence detector at an excitation wavelength of 300 nm and an emission wavelength of 435 nm. The intraday precision, measured as a coefficient of variation at 25 ng/mL, was 6.7%. The interday precision was 7.3%.

Blood lead concentration. To test whether the association with MRSI results was specific to blood concentrations of harmane, we also examined data that had been collected on blood lead concentrations because lead can produce tremor. As described previously, blood lead concentration was analyzed in our subjects using graphite furnace atomic absorption spectrophotometry. These analyses were performed blinded to clinical information and MRSI data.

Statistical analyses. All analyses were performed in SPSS version 13.0. To assess group differences in continuous variables (e.g., NAA/tCR by gender), Student t tests were used. Pearson correlation coefficients were used to assess associations between continuous variables (e.g., NAA/tCR by age). Because blood concentrations of harmane and lead typically are not normally distributed, these were log transformed (log10). Our study hypothesis was to test the association between log blood harmane concentration and cerebellar NAA/tCR. To test this hypothesis, we used a linear regression analysis in which log blood harmane concentration was the independent variable and cerebellar NAA/tCR was the dependent variable while adjusting for various covariates (e.g., age and gender). In a series of secondary analyses, we also assessed associations between blood harmane concentration and NAA/tCR in four other brain regions and blood concentration of another neurotoxin (lead) and cerebellar NAA/tCR.

RESULTS The mean ± SD cerebellar NAA/tCR was 1.52 ± 0.41 (range = 1.06 to 2.29). The mean ± SD log blood harmane concentration was 0.70 ± 0.52 g 10/mL (range = 0.13 to 1.72 g 10/mL). Cerebellar NAA/tCR was strongly associated with age in years (r = −0.70, p = 0.01), as has been reported previously. It was also associated with gender (1.91 ± 0.45 [4 men] vs 1.33 ± 0.21 [8 women], t = 3.16, p = 0.01), but not with race (1.58 ± 0.42 [10 white] vs 1.23 ± 0.25 [2 non-white], t = 1.11, p = 0.29) or use of a medication to treat tremor (1.36 ± 0.12 [2 yes] vs 1.55 ± 0.44 [10 no], t = 0.60, p = 0.56). MRSI was performed a mean of 8.2 ± 7.9 months after the blood collection. The length of the latency between these two procedures was not associated with log blood harmane concentration (r = −0.18, p = 0.58) or with cerebellar NAA/tCR (p = 0.21, p = 0.52).

In an initial unadjusted model, the correlation (r) between log blood harmane concentration and cerebellar NAA/tCR was −0.32 (p = 0.30). However, this analysis did not take age, a strong confounding factor, into consideration. Hence, in a linear regression model that adjusted for a single variable, age, log blood harmane concentration was a predictor of cerebellar NAA/tCR (beta = −0.34, p = 0.05) independent of the confounding effects of age. In a similar linear regression model that adjusted for both confounders (age and gender), log blood harmane concentration was a predictor of cerebellar NAA/tCR: beta = −0.41, p = 0.009. For every 1 g 10/mL unit increase in log blood harmane concentration, there was, on average, a 0.41 unit decrease in cerebellar NAA/tCR. Finally, in a linear regression model that adjusted for age, gender, race, tremor medication, and latency between blood collection and MRSI, higher log blood harmane concentration was associated with lower cerebellar NAA/tCR: beta = −0.40, p = 0.02.

The association between log blood harmane concentration and brain NAA/tCR was particular to the primary ROI (cerebellar cortex); it was not observed for the other four secondary ROIs (table). Furthermore, the association was specific to blood concentration of harmane and not blood lead concentration. In fact, higher concentrations of blood lead were associated with higher rather
DISCUSSION In this study of patients with ET, higher blood harmane concentration was associated with lower cerebellar NAA/tCR. For every 1 g·10⁻¹⁹/mL unit increase in log blood harmane concentration, there was, on average, a 0.41 unit decrease in cerebellar NAA/tCR (i.e., higher concentrations of neurotoxin were associated with greater metabolic dysfunction). This association between blood harmane concentration and brain NAA/tCR was particular to the primary ROI, namely, the cerebellar cortex; it was not present in the other brain ROIs we examined. Furthermore, the association was specific to harmane and was not observed as a feature of another neurotoxin, lead, which we also studied.

NAA is an amino acid present exclusively in the cytosol of neurons and a reduction in NAA is thought to be an indicator of neuronal dysfunction or loss. In previous studies of ET, reductions in NAA were demonstrated in the cerebellum, indicating neuronal dysfunction or loss in this brain region. This neuronal dysfunction/loss was linked in the current study with blood concentrations of harmane. Harmane is a HCA and, more specifically, a beta-carboline alkaloid. Other members of its chemical class (e.g., harmaline, harmine) are highly neurotoxic, and it has been known for more than 100 years that administration of these chemicals to a wide variety of laboratory animals (e.g., mice, cats, monkeys) produces an intense and generalized action tremor that resembles ET. The harmaline model, indeed, is the traditional animal model for ET and new therapies are tested using exposed animals. Beta carboline alkaloids, including harmane, are present in a variety of foods (e.g., plants and meats) and certain cooking practices (e.g., char-broiling meats) increase the concentrations of these chemicals. Once they are ingested and enter the systemic circulation, these chemicals cross the blood–brain barrier. Members of this class of chemicals are able to produce toxic damage with marked destruction of cerebellar Purkinje cells. The present study, in suggesting a link between harmane and brain changes in patients with ET, represents a beginning. Additional studies are needed to further test this association. For example, if postmortem tissue becomes available, one can assess whether cerebellar harmane concentration is elevated in ET cases relative to controls and whether higher concentrations are associated with greater postmortem changes in the cerebellum. Such tissue is not available in any of the 12 cases we have studied.

The mechanisms for the elevated blood harmane concentration in ET cases are not entirely clear. However, investigators have postulated that, in mammals, dietary sources of harmane probably play only a minor role and that endogenous production is the major contributor to blood harmane concentrations. Studies have demonstrated that plasma concentrations of harmane do not change significantly after meals and that they remain stable over several weeks. In a study of patients with liver transplant, while blood harmane concentrations dropped significantly posttransplant, they then remained remarkably stable over the ensuing 36-month period, further confirming that blood levels are primarily a reflection of the body’s metabolic capacity and that they may remain relatively stable for long periods of time. In a small sample of 86 of our own cases on whom we have data on the precise time of phlebotomy, we have found no correlation between log blood harmane concentration and time of blood draw (r = 0.10, p = 0.36), suggesting that the concentration is independent of time of day. Our own recent dietary data also suggest that genetic-metabolic factors may be more important than dietary intake. In a study of the blood harmane and diet, while blood harmane concentration was elevated in ET cases, we did not find any differences in dietary protein consumption between patients with ET and controls. In that study, cases and controls consumed similar amounts of animal protein (50.2 ± 19.6 vs 49.4 ± 19.1 g/day, p = 0.74). In aggregate, current data suggest that blood harmane concentrations are likely to be chronically (i.e., metabolically) elevated in patients with ET, which would mean...
that the latency in the present study between the
determination of blood harmarne concentrations
and the MRSI results is not likely to have been an
important issue. To further put this to the test,
however, future studies could provide serial mea-
sures of these concentrations.

This study has limitations. With 12 patients,
the sample size was modest and future studies
should include larger numbers of patients. De-
spite this, the association we observed reached
significant levels, indicating that we did not com-
mit a Type II error (i.e., failure to reject the null
hypothesis when it is false). Furthermore, the
specificity of the association (i.e., the association
was only observed between one toxin and one
particular brain region) indicates that it is un-
likely that our results represent a Type I error
(i.e., rejection of the null hypothesis when it is
true). A second limitation is that we approxi-
imated the concentration of NAA relative to tCR
(i.e., NAA/tCR). We did not perform absolute
quantitation of NAA. An advantage of absolute
quantitation of NAA is that the observed changes
in NAA only reflect changes in NAA rather than
changes in either NAA or tCR, as is the case when
dealing with ratios (NAA/tCR). Third, in most
instances, phlebotomy and MRSI were not per-
formed on the same day, with, on average, an 8.2-
month latency between the two measures. While
this may have affected our results, we adjusted for
this latency in our regression models, and it did
not explain the observed association between log
blood harmarne concentration and cerebellar
NAA/tCR. Finally, although cooking and dietary
practices may influence the intake of harmarne
and other beta-carboline alkaloids, one should
also consider other factors. One is that gastroin-
testinal bacteria may generate these chemicals
(e.g., tetrahydroharman).40 Also, blood concen-
trations of these chemicals could be dependent on
differences in cytochrome P450 enzymes.41

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