Blood harmane concentrations and dietary protein consumption in essential tremor

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Abstract—*Background:* β-Carboline alkaloids (e.g., harmane) are highly tremorogenic chemicals. Animal protein (meat) is the major dietary source of these alkaloids. The authors previously demonstrated that blood harmane concentrations were elevated in patients with essential tremor (ET) vs controls. Whether this difference is due to greater animal protein consumption by patients or their failure to metabolize harmane is unknown. *Objective:* The aim of this study was to determine whether patients with ET and controls differ with regard to 1) daily animal protein consumption and 2) the correlation between animal protein consumption and blood harmane concentration. *Methods:* Data on current diet were collected with a semiquantitative food frequency questionnaire and daily calories and consumption of animal protein and other food types was calculated. Blood harmane concentrations were log-transformed (logHA). *Results:* The mean logHA was higher in 106 patients than 161 controls (0.61 ± 0.67 vs 0.43 ± 0.72 g^{-10}/mL, $p = 0.035$). Patients and controls consumed similar amounts of animal protein (50.2 ± 19.6 vs 49.4 ± 19.1 g/day, $p = 0.74$) and other food types (animal fat, carbohydrates, vegetable fat) and had similar caloric intakes. In controls, logHA was correlated with daily consumption of animal protein ($r = 0.24$, $p = 0.003$); in patients, there was no such correlation ($r = -0.003$, $p = 0.98$). *Conclusions:* The similarity between patients and controls in daily animal protein consumption and the absence of the normal correlation between daily animal protein consumption and logHA in patients suggests that another factor (e.g., a metabolic defect) may be increasing blood harmane concentration in patients.

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β-Carboline alkaloids (BCAs), including harmane, harmaline, and others, are a group of naturally occurring chemicals that cause action tremor.[1,2] Although BCAs are produced endogenously in the human body,[3,4] the quantity derived from exogenous (dietary) sources may be fifty times greater than that which results from endogenous production.[5] BCAs are primarily found in cooked animal protein (beef, chicken, pork, fish) at ng/g concentrations.[6-8] Like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, with which they have a structural similarity,[9] BCAs are highly neurotoxic, and administration of BCAs to a wide variety of laboratory animals produces severe action tremor that resembles essential tremor (ET).[10] BCA administration is the major animal model for ET and new therapies are tested using exposed animals.[11] Human volunteers acutely exposed to large doses of BCAs display a coarse tremor.[12,13] BCA-induced tremor shares many features with ET including its principal clinical features, drug-response characteristics,[14-19] and underlying brain changes (cerebellar changes have been noted in physiologic and imaging studies of patients with ET; similarly, BCAs damage olivary-cerebellar pathways).[10,14,18,20-23]

The etiology of ET is poorly understood[24] and a better understanding of disease etiology and mechanisms has the potential to modify or prevent a disorder that affects as much as 4% of the adult population.[25] We previously demonstrated that blood concentrations of the BCA harmane were elevated in patients with ET vs controls.[26] Whether this is due to increased animal protein consumption in patients or their failure to metabolize harmane is not known. We sought to determine 1) whether patients with ET consume more animal protein than controls and 2) whether animal protein consumption is correlated differently with blood harmane concentrations in controls than in patients. We also evaluated whether elevated harmane concentrations, which have been associated with increased odds of ET,[26] are the result of dietary factors that differ between patients with ET and controls or due to an inherent metabolic difference between the two.

Methods. *Participants.* All participants were enrolled in a study of the environmental epidemiology of ET. Patients with ET were patients seen at the Neurologic Institute of New York, Columbia University Medical Center (CUMC). They were identified from a computerized database listing names and diagnoses of all

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patients billed within the past 3 years supplemented by a computerized database at the Center for Parkinson's Disease and Other Movement Disorders, CUMC, which listed names and diagnoses of patients seen within the past 10 years. All patients had received a diagnosis of ET from their treating neurologist at the Institute. All patients with ET were selected for enrollment. Office records were reviewed, and patients with diagnoses or physical signs of dystonia, Parkinson disease (PD), or spinocerebellar ataxia were excluded.

Controls were identified from the New York Tristate area using random digit telephone dialing. Patients and controls were ascertained from the same source population in the New York Tristate region (i.e., controls were selected from the same set of zip codes in New York, New Jersey, and Connecticut as were the patients with ET). The majority of these controls also received their health care at the same medical center as did the patients with ET (CUMC). These controls were frequency matched to CUMC patients on 5-year age strata, gender, and ethnicity. Control recruitment proceeded more rapidly than patient recruitment, resulting in a larger number of controls than patients. To date, 513 subjects (232 patients with ET and 281 controls) have been enrolled and data entered on 463. Of these 463, 267 (57.7%) (106 patients with ET and 161 controls) were included in these analyses. The remaining 196 were excluded for the following reasons: phlebotomy was unsuccessful or quantity of blood was not sufficient for blood harmane analysis (n = 43), subjects did not fulfill diagnostic criteria for ET (e.g., they had PD, psychogenic tremor, or very mild action tremor; n = 26), blood specimen was not analyzed for harmane concentration because laboratory was in the process of moving locations (n = 47), dietary data were not completed fully (n = 39), and other data were missing (n = 41). The 196 who were excluded did not differ from the 267 who were included in terms of gender, ethnicity, or education but they were, on average, 4 years older.

Clinical evaluation. All subjects were evaluated in person by a trained tester. The tester was trained for 1 month by a neurologist (E.D.L.) to administer clinical questionnaires and to perform a videotaped examination. Most evaluations were home visits and therefore were performed in the late morning or early afternoon, making fasting levels impractical. Data suggest that plasma concentrations of harmane do not change significantly as long as 8 hours after meals.[27]

The tester collected demographic, clinical, and family history information, including data on number of rooms in the home (a socioeconomic indicator). Data on current diet were collected using a Willett Semi-Quantitative Food-Frequency Questionnaire. This 20-minute food-frequency questionnaire[28] included questions on frequency of current consumption of 61 foods and on the use of vitamins and mineral supplements. Food frequency data may be used to compute mean daily intake of total protein, animal protein, animal fat, vegetable fat, and carbohydrates (each in grams), calories (kilocalories), ethanol (grams), and caffeine (milligrams). The questionnaire has shown good reliability and validity related to recent nutrient intake.[28] Current cigarette smoking status was assessed in each subject.

Weight and height were assessed using a balance scale designed for field surveys (Scale-Tronix 5600, White Plains, NY) and a movable anthropometer (GPM Martin Type, Pfister Inc., Carlstadt, NJ). Body mass index was calculated as weight in kilograms divided by the square of height in meters.

The tester videotaped a tremor examination in all participants,[29,30] and each of 12 videotaped action tremor items was rated (E.D.L.) on a scale from 0 to 3, resulting in a total tremor score (range = 0 to 36 [maximum]). The rater also noted the presence or absence of head tremor on the videotaped examination. The diagnosis of ET was confirmed by the rater using published diagnostic criteria (moderate amplitude tremor during three activities or a head tremor) and diagnostic gradations of possible, probable, and definite ET.[29,30] None of the patients or control subjects had PD or dystonia.

Blood harmane concentrations. During the clinical evaluation, phlebotomy was performed. Blood concentrations of harmane were measured blinded to any clinical information, including age, gender, and diagnosis. Samples were run in batches of 10, containing, when possible an approximately equal number (four to six) of patients and control specimens. A novel high-performance liquid chromatography (HPLC) method for quantifying harmane in blood has been reported.[26,31,32] 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, vol/vol), 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%.[32]

Statistical analyses. Statistical analyses were performed in SPSS (Version 11.0). The empirical distribution of harmane was positively skewed. Using a one-sample Kolmogorov–Smirnov test, we tested whether harmane concentration was normally distributed. Rejection of the null hypothesis (i.e., $p < 0.05$) is consistent with a nonnormal distribution. Harmane was not normally distributed in patients or controls (Kolmogorov–Smirnov $z = 1.54$, $p = 0.02$ in patients, and $z = 1.62$, $p = 0.01$ in controls). Therefore, blood harmane concentrations were logarithmically transformed (logHA) and patient-control differences compared using Student t tests. Chi-square tests were used to analyze proportions and Student t tests to examine group differences in continuous variables (table 1). Pearson (r) correlation coefficients were used to assess correlations between continuous variables.

In a logistic regression analysis, we tested whether the outcome, diagnosis (patient vs control), differed with respect to logHA. We also adjusted for age in these analyses; these analyses resulted in odds ratios (OR) with 95% CI per unit change in logHA. For some analyses, we stratified study subjects by tremor severity and diagnosis into five categories: 1) control subjects with no detectable tremor (total tremor score = 0), 2) control subjects with very mild tremor that was in the range of normal, 3) subjects with possible ET, 4) subjects with probable ET, and 5) subjects with definite ET (table 2). We then tested whether logHA was associated with tremor severity/diagnosis category in a linear regression analysis (test for trend; see table 2).

Daily consumption of food types was compared in patients and controls using Student t tests (table 3). In logistic regression analyses that adjusted for daily calories, age in years, body mass index, and years at current address, we tested whether patients vs controls differed with respect to either daily total protein intake or daily animal protein intake. We then tested whether daily protein consumption was associated with tremor severity/diagnosis category in a linear regression analysis (test for trend; see table 2) and, in a second linear regression analysis, tested whether daily animal protein consumption was associated with tremor severity-diagnosis category (test for trend; see table 2). Correlations between food types and logHA were examined separately in patients and controls using Pearson correlation coefficients (r, table 4). In linear regression analyses that controlled for calories, age, body mass index, and years at current address, we tested whether logHA was associated with daily consumption of total protein in one model and with daily consumption of animal protein in a second model.

Using pilot data on the expected mean daily protein consumption in controls (64.0 ± 20.2 g) and assuming $\alpha = 0.05$, our study, with approximately 100 patients with ET and approximately 150 controls, had 95.6% power to detect as little as a 15% difference in daily protein consumption between patients and controls. Assuming that $\alpha = 0.01$ resulted in 86.1% power. With the addition of data on 114 previously unreported subjects, the current sample represents a 65% increase in sample size from that of our earlier report; we note that the earlier report did not contain data on animal protein consumption or its correlation with logHA in patients vs controls.[26]

Results. *LogHA in patients vs controls.* There were 106 patients and 161 controls. The mean logHA was higher in patients than controls (0.61 ± 0.67 g^{-10}/mL vs 0.43 ± 0.72 g^{-10}/mL, $p = 0.035$). The patients and controls had similar demographic characteristics, except for age (see table 1); patients were on average 6 years older than controls.

We examined the factors that were associated with log HA in controls; logHA was associated with daily caloric consumption ($r = 0.16$, $p = 0.045$). LogHA was not associated with age in years ($r = 0.03$, $p = 0.67$), or other potential confounders (gender, ethnicity, current smokers vs

Table 1 *Demographic and clinical characteristics of patients with ET vs controls*

	Patients with ET, n = 106	Controls, n = 161	Significance
Age, y	69.7 ± 13.6	63.8 ± 12.6	t = 3.62, p < 0.001
Education, y	15.2 ± 3.3	15.4 ± 3.3	t = 0.35, p = 0.73
No. of rooms in home	5.7 ± 2.3	5.7 ± 2.3	t = 0.21, p = 0.83
Years at current address	21.9 ± 16.3	22.1 ± 14.6	t = 0.13, p = 0.90
Sex, n (% female)	62 (58.5%)	93 (57.8%)	χ^2 = 0.01, p = 0.91
Ethnicity			χ^2 = 4.42, p = 0.22
Non-Hispanic white	100 (94.3%)	142 (88.2%)	
Non-Hispanic African-American	3 (2.9%)	6 (3.7%)	
Hispanic	3 (2.9%)	8 (5.0%)	
Other	0 (0.0%)	5 (3.1%)	
Current smokers	8 (7.6%)	16 (9.9%)	χ^2 = 0.45, p = 0.50
Total tremor score	20.6 ± 7.6	3.8 ± 2.8	t = 21.50, p < 0.001
Tremor duration, y	22.4 ± 18.3	Not applicable	Not applicable

ET = essential tremor.

nonsmokers, years of education, number of rooms in home, years at current address, body mass index, daily caffeine consumption in milligrams, or daily ethanol consumption in grams [p values ranging from 0.27 to 0.97, with the exception of daily ethanol consumption in grams in which $p = 0.14$]).

In a logistic regression analysis, logHA was associated with diagnosis (OR 1.54, 95% CI: 1.07 to 2.22, $p = 0.02$). After adjusting for age in a logistic regression analysis, OR 1.43, 95% CI: 0.98 to 2.41, $p = 0.07$. To further assess the association between logHA and diagnosis, we stratified all 267 study subjects by tremor severity and diagnosis into five categories. When comparing categories 1 through 5, there was a progressive increase in logHA (test for trend, $p = 0.008$; see table 2 and figure).

Aim 1: Do patients with ET consume more animal protein than controls? Patients and controls consumed similar daily quantities of total protein, animal protein, animal fat, vegetable fat, carbohydrates, calories, and ethanol (see table 3). Patients consumed fewer milligrams of caffeine per day than did controls (see table 3).

We examined the factors that were associated with daily animal protein consumption in controls; daily animal protein consumption was associated with daily caloric consumption ($r = 0.65$, $p < 0.001$) and body mass index ($r = 0.25$, $p = 0.002$) and marginally associated with age ($r = 0.15$, $p = 0.06$) and years at current address ($r = 0.14$, $p = 0.07$) but not associated with gender, ethnicity, current smokers vs nonsmokers, years of education, number of rooms in home, daily caffeine consumption in milligrams, or daily ethanol consumption in grams (p values ranging from 0.19 to 0.98).

In a logistic regression analysis that adjusted for potential confounders (daily calories, age in years, body mass index, and years at current address), daily total protein intake did not differ between patients and controls (OR 1.00, 95% CI: 0.98 to 1.02, $p = 0.88$). In a similar adjusted analysis, daily animal protein intake did not differ between patients and controls (OR 1.006, 95% CI: 0.99 to 1.02, $p = 0.48$).

We stratified study subjects by tremor severity and diagnosis into five categories. When comparing categories 1 through 5, there was no increase in daily consumption of total protein (test for trend, $p = 0.54$; see table 2) or in daily consumption of animal protein (test for trend, $p = 0.93$; see table 2).

Aim 2: Correlation between animal protein consumption and LogHA (controls vs patients). Among controls, logHA was associated with daily consumption of total protein ($r = 0.23$, $p = 0.005$), animal protein ($r = 0.24$, $p = 0.003$),

Table 2 *Association between five tremor severity/diagnosis categories and logHA, total daily protein consumption, and total daily animal protein consumption*

	n	Total tremor score	LogHA	Total daily protein consumption, g	Total daily animal protein consumption, g
1: Controls with no tremor	16	0.0 ± 0.0	0.21 ± 0.61	65.3 ± 22.1	46.8 ± 17.0
2: Controls with mild tremor	145	4.3 ± 2.6	0.45 ± 0.73	66.2 ± 22.6	49.7 ± 19.4
3: Possible ET	15	14.8 ± 4.6	0.48 ± 0.64	67.8 ± 19.0	52.8 ± 18.3
4: Probable ET	53	17.5 ± 6.0	0.56 ± 0.67	66.7 ± 22.5	52.5 ± 20.9
5: Definite ET	38	27.1 ± 5.9	0.74 ± 0.69	62.3 ± 20.4	46.1 ± 18.0
Test for trend		p < 0.001	p = 0.008	p = 0.54	p = 0.93

ET = essential tremor.

Table 3 *Daily dietary consumption of food types, including total protein and animal protein, in patients with ET vs controls*

Daily dietary consumption	Patients with ET	Controls	Significance
Total protein, g	65.2 ± 21.2	66.2 ± 22.5	t = 0.33, p = 0.74
Animal protein, g	50.2 ± 19.6	49.4 ± 19.1	t = 0.33, p = 0.74
Animal fat, g	28.0 ± 11.7	29.0 ± 13.9	t = 0.61, p = 0.54
Vegetable fat, g	19.2 ± 9.4	21.2 ± 12.0	t = 1.46, p = 0.15
Carbohydrates, g	177.7 ± 61.0	177.9 ± 80.5	t = 0.02, p = 0.98
Calories, kcal	1,407.4 ± 369.6	1,435.8 ± 498.2	t = 0.52, p = 0.61
Ethanol, g	6.4 ± 9.4	6.3 ± 10.2	t = 0.07, p = 0.94
Caffeine, mg	128.8 ± 140.2	242.4 ± 215.3	t = 5.13, p < 0.001

ET = essential tremor.

animal fat ($r = 0.22$, $p = 0.006$), and calories ($r = 0.16$, $p = 0.045$) but not with vegetable fat, carbohydrates, ethanol, or caffeine (see table 4). In linear regression analyses that controlled for calories, age, body mass index, and years at current address, logHA in controls was associated with daily consumption of total protein ($\beta = 0.009$, $p = 0.03$) and with daily consumption of animal protein ($\beta = 0.009$, $p = 0.04$).

In patients, logHA was not associated with daily consumption of total protein, animal protein, or any of the other food types (see table 4). In linear regression analyses that controlled for calories, age, body mass index, and years at current address, logHA in patients was not associated with daily consumption of total protein ($\beta = -0.0008$, $p = 0.85$) or with daily consumption of animal protein ($\beta = 0.0001$, $p = 0.81$).

Discussion. Animal protein consumption was similar in patients with ET and controls. Moreover, in normal controls, daily animal protein consumption was correlated with blood concentration of harmane; individuals with higher reported dietary intake of animal protein had higher blood harmane concentrations. In patients with ET, this normal correlation between diet and blood concentration seemed to be absent. These data suggest that a factor other than increased dietary protein intake is responsible for the elevated blood harmane concentration in patients with ET and that the normal correlation between dietary protein intake and blood harmane concentration is disturbed. Mechanisms for this disturbance could be increased endogenous production of harmane in patients with ET or difficulty in metabolizing harmane in these patients. Therefore, future studies should examine the role of BCA metabolism in the etiology of ET.

Little is known about the normal endogenous production of BCAs in humans; more is known about their metabolism. BCAs are a type of heterocyclic amine and, in terms of their metabolism, food-derived heterocyclic amines undergo *N*-oxidation by the hepatic cytochrome P-450 system, resulting in an *N*-hydroxy metabolite.[31,33-35] The most active P-450 isoform, CYP1A2, is encoded by the *CYP1A2* gene, which localizes to human chromosome 15q22-qter.[33,35,36] In humans, the *N*-hydroxy metabolite is further metabolized by acetyltransferases and, most importantly, *N*-acetyltransferase 2 (NAT2), a 30-kd protein.[5,33-35,37,38] The gene encoding this enzyme, denoted NAT2, localizes to human chromosome 8p22. Individuals with slow acetylation capacity may have increased susceptibility to disease due to the accumulation of toxic metabolites.[39,40] It is not known whether patients with ET and controls differ in the

Table 4 *Correlations between daily dietary consumptions of protein and other food types with logHA in patients with ET and controls*

	Patients with ET	Controls
Total protein	r = 0.03, p = 0.76	r = 0.23, p = 0.005
Animal protein	r = −0.003, p = 0.98	r = 0.24, p = 0.003
Animal fat	r = −0.07, p = 0.46	r = 0.22, p = 0.006
Vegetable fat	r = −0.15, p = 0.13	r = 0.04, p = 0.59
Carbohydrates	r = 0.07, p = 0.47	r = 0.11, p = 0.16
Calories	r = 0.001, p = 0.99	r = 0.16, p = 0.045
Ethanol	r = 0.02, p = 0.83	r = −0.12, p = 0.14
Caffeine	r = −0.13, p = 0.19	r = −0.07, p = 0.41

ET = essential tremor.

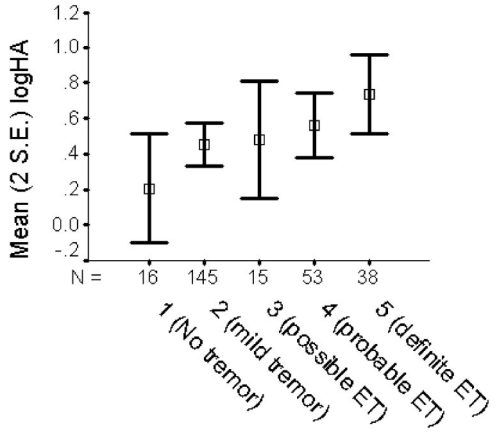

Figure. Mean logHA in five tremor severity/diagnosis categories. Test for trend, p = 0.008.

distribution of polymorphisms in the CYP1A2 or NAT2 genes, but this is worthy of future study.

As reported previously,[41] patients with ET consumed less caffeine than did controls, which is likely to be a dietary modification in response to tremor. The intake of ethanol did not differ between patients with ET and controls. As noted previously,[41] this may be a function of the age of our sample; many older individuals refrain from the use of ethanol for a variety of reasons, including its contraindication in the setting of numerous medications; as a result, alcohol consumption diminishes with age.

Although dietary meat is considered the major source of BCAs such as harmane,[6-8] we are unaware of a previous study that has demonstrated a correlation between meat consumption and a biologic measure of BCAs (i.e., blood harmane concentration). This report further strengthens the notion that biologic burden of BCAs is related to diet and, more specifically, to animal protein consumption.[6-8]

We did not assess fasting levels of harmane, although dietary intake of meats on the day of phlebotomy would only have increased the association between dietary intake and blood harmane concentration, making it more likely that we would have found a significant correlation between the two in patients with ET. Also, we did not assess liver function, variability in the cytochrome P-450 system, or renal function to see whether these factors, which could influence the metabolism of harmane, differed in patients and controls. We assessed current rather than predisease dietary intake and current blood harmane concentrations. Assessment of predisease dietary intake is more relevant for etiologic studies of ET and would allow investigators to determine whether alterations in diet or in harmane metabolism preceded the onset of disease rather than vice versa. Finally, our patients with ET were not ascertained directly from the population, so that it is possible that these patients were selected for a more severe form of the disease.

References

1. Zetler G, Singbartl G, Schlosser L. Cerebral pharmacokinetics of tremor-producing harmala and iboga alkaloids. Pharmacology 1972;7:237–248.
2. Sakai S. Chemical studies of indole alkaloids. Yakugaku Zasshi 1995;115:351–369.
3. Wakabayashi K, Totsuka Y, Fukutome K, Oguri A, Ushiyama H, Sugimura T. Human exposure to mutagenic/carcinogenic heterocyclic amines and comutagenic beta-carbolines. Mutat Res 1997;376:253–259.
4. Ushiyama HK, Oguri Y, Totsuka H, Itoh H, Sugimura T, Wakabayashi K. Norharman and harman in human urine. Proc Jpn Acad 1995; 71B:57–60.
5. Pfau W, Skog K. Exposure to beta-carbolines norharman and harman. J Chromatogr B 2004;802:115–126.
6. Gross GA, Turesky RJ, Fay LB, Stillwell WG, Skipper PL, Tannenbaum SR. Heterocyclic aromatic amine formation in grilled bacon, beef and fish and in grill scrapings. Carcinogenesis 1993;14:2313–2318.
7. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, Felton JS. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis 1995;16:39–52.
8. Skog K. Cooking procedures and food mutagens: a literature review. Food Chem Toxicol 1993;31:655–675.
9. De Meester C. Genotoxic potential of beta-carbolines: a review. Mutat Res 1995;339:139–153.
10. Du W, Aloyo VJ, Harvey JA. Harmaline competitively inhibits [3H]MK-801 binding to the NMDA receptor in rabbit brain. Brain Res 1997;770:26–29.
11. Krahl SE, Martin FC, Handforth A. Vagus nerve stimulation inhibits harmaline-induced tremor. Brain Res 2004;1011:135–138.
12. Lewin L. Untersuchungen uber Banisteria caapi Sp. Arch Exp Pathol Pharmacol 1928;129:133–149.
13. Pennes HH, Hoch PH. Psychotomimetics, clinical and theoretical considerations: harmine, Win-2299 and Nalline. Am J Psychiatry 1957;13:885–892.
14. Milner TE, Cadoret G, Lessard L, Smith AM. EMG analysis of harmaline-induced tremor in normal and three strains of mutant mice with Purkinje cell degeneration and the role of the inferior olive. J Neurophysiol 1995;73:2568–2577.
15. Trouvin JH, Jacqmin P, Rouch C, Lesne M, Jacquot C. Benzodiazepine receptors are involved in taberanthine-induced tremor: in vitro and in vivo evidence. Eur J Pharmacol 1987;140:303–309.
16. Fuentes JA, Longo VG. An investigation on the central effects of harmine, harmaline and related B-carbolines. Neuropharmacology 1971;10:15–23.
17. Cross AJ, Misra A, Sandilands A, Taylor MJ, Green AR. Effect of chlormethiazole, dizocilpine and pentobarbital on harmaline-induced increase of cerebellar cyclic GMP and tremor. Psychopharmacology 1993;111:96–98.
18. Sinton CM, Krosser BI, Walton KD, Llinas RR. The effectiveness of different isomers of octanol as blockers of harmaline-induced tremor. Pflugers Arch 1989;414:31–36.
19. Rappaport MS, Gentry RT, Schneider DR, Dole VP. Ethanol effects on harmaline-induced tremor and increase of cerebellar cyclic GMP. Life Sci 1984;34:49–56.
20. Deuschl G, Bergman H. Pathophysiology of nonparkinsonian tremors. Mov Disord 2002 17(suppl 3):S41–S48.
21. Deuschl G, Wenzelburger R, Loffler K, Raethjen J, Stolze H. Essential tremor and cerebellar dysfunction clinical and kinematic analysis of intention tremor. Brain 2000;123:1568–1580.
22. O'Hearn E, Molliver ME. Degeneration of Purkinje cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline. Neuroscience 1993;55:303–310.
23. Robertson HA. Harmaline-induced tremor: the benzodiazepine receptor as a site of action. Eur J Pharmacol 1980;67:129–132.
24. Louis ED. Etiology of essential tremor: should we be searching for environmental causes? Mov Disord 2001;16:822–829.
25. Dogu O, Sevim S, Camdeviren H et al. Prevalence of essential tremor: door-to-door neurological exams in Mersin Province, Turkey. Neurology 2003;61:1804–1807.
26. Louis ED, Zheng W, Jurewicz EC et al. Elevation of blood β-carboline alkaloids in essential tremor. Neurology 2002;59:1940–1944.
27. Rommelspacher H, Schmidt LG, May T. Plasma norharman (β-carboline) levels are elevated in chronic alcoholics. Alcohol Clin Exp Res 1991;15:553–559.
28. Willett WC, Sampson L, Stampfer MJ et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
29. Louis ED, Ottman RA, Ford B et al. The Washington Heights Essential Tremor Study: Methodologic issues in essential-tremor research. Neuroepidemiology 1997;16:124–133.
30. Louis ED, Ford B, Lee H, Andrews H. Does a screening questionnaire for essential tremor agree with the physician's examination? Neurology 1998;50:1351–1357.
31. Guan Y, Louis ED, Zheng W. Toxicokinetics of tremorogenic natural products, harmane and harmine, in male Sprague-Dawley rats. J Toxicol Environ Health 2001;64:101–116.
32. Zheng W, Wang S, Barnes LF, Guan Y, Louis ED. Determination of harmane and harmine in human blood using reversed-phase high-performance liquid chromatography and fluorescence detection. Anal Biochem 2000;279:125–129.
33. Lang NP, Butler MA, Massengill J et al. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. Cancer Epidemiol Biomarkers Prev 1994;3:675–682.
34. Sachse C, Smith G, Wilkie MJV et al. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis 2002;23:1839–1849.
35. Pavanello S, Simioli P, Mastrangelo G. Role of metabolic polymorphisms NAT2 and CYP1A2 on urinary mutagenicity after a pan-fried hamburger meal. Food Chem Toxicol 2002;40:1139–1144.
36. Gooderham NJ, Murray S, Lynch AM et al. Food-derived heterocyclic amine mutagens: variable metabolism and significance in humans. Drug Metab Dispos 2001;29:529–534.
37. Barrett JH, Smith G, Waxman R et al. Investigation of interaction between *N*-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. Carcinogenesis 2003;24:275–282.

38. Ishibe N, Sinha R, Hein DW et al. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. Pharmacogenetics 2002;12:145–150.
39. Agundez JAG, Jimenez-Jimenez FJ, Luengo A et al. Slow allotypic variants of the NAT2 gene and susceptibility to early-onset Parkinson's disease. Neurology 1998;51:1587–1592.
40. Bandmann O, Vaughan JR, Holmans P, Marsden CD, Wood NW. Detailed genotyping demonstrates association between the slow acetylator genotype for *N*-acetyltransferase 2 (NAT2) and familial Parkinson's disease. Mov Disord 2000;15:30–35.
41. Louis ED, Jurewicz EC, Applegate L, Luchsinger JA. Factor-Litvak P, Parides M. Semi-quantitative study of current coffee, caffeine and ethanol intake in essential tremor cases and controls. Mov Disord 2004;19: 499–504.