Published in final edited form as:

Neurotoxicology. 2011 March ; 32(2): 227–232. doi:10.1016/j.neuro.2010.12.002.

Blood Harmane, Blood Lead, and Severity of Hand Tremor: Evidence of Additive Effects

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Abstract

Background—Tremor is a widespread phenomenon in human populations. Environmental factors are likely to play an etiological role. Harmane (1-methyl-9H-pyrido[3,4-β]indole) is a potent tremor-producing β-carboline alkaloid. Lead is another tremor-producing neurotoxicant. The effects of harmane and lead with respect to tremor have been studied in isolation.

Objectives—We tested the hypothesis that tremor would be particularly severe among individuals who had high blood concentrations of both of these toxicants.

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Statistical Analyses: The statistical analyses were conducted by Dr. Louis.

5. Conflict of Interests Statement: The authors declare that there are no conflicts of interest.

Financial Disclosure: Elan D. Louis was funded by R01 NS39422, P30 ES09089, RR00645 (General Clinical Research Center) from the National Institutes of Health (Bethesda, MD and Research Triangle, NC). Pam Factor-Litvak was funded by R01 ES12231, R01 ES017024 from the National Institutes of Health (Research Triangle, NC). Joseph H Graziano was funded by P42 ES10349 from the National Institutes of Health (Research Triangle, NC). Wei Zheng was funded by R01 NS39422 and R01 ES008146 from the National Institutes of Health (Research Triangle, NC).

The National Institutes of Health played no role in the study design, the collection of data, the analysis and interpretation of data, the writing of the paper or in the decision to submit the paper for publication. The authors were free to design, conduct, interpret, and publish research and this was not compromised by the National Institutes of Health.

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Methods—Blood concentrations of harmane and lead were each quantified in 257 individuals (106 essential tremor cases and 151 controls) enrolled in an environmental epidemiological study. Total tremor score (range = 0 – 36) was a clinical measure of tremor severity.

Results—The total tremor score ranged from 0 – 36, indicating that a full spectrum of tremor severities was captured in our sample. Blood harmane concentration correlated with total tremor score ($p = 0.007$), as did blood lead concentration ($p = 0.045$). The total tremor score was lowest in participants with both low blood harmane and lead concentrations (8.4 ± 8.2), intermediate in participants with high concentrations of either toxicant (10.5 ± 9.8), and highest in participants with high concentrations of both toxicants (13.7 ± 10.4) ($p = 0.01$).

Conclusions—Blood harmane and lead concentrations separately correlated with total tremor scores. Participants with high blood concentrations of both toxicants had the highest tremor scores, suggesting an additive effect of these toxicants on tremor severity. Given the very high population prevalence of tremor disorders, identifying environmental determinants is important for primary disease prevention.

Keywords

tremor; essential tremor; neurology; harmane; lead; toxicant; epidemiology; etiology

1. Introduction

Tremor is a widespread phenomenon within human populations, with more than 95% of normal adults exhibiting some clinically-evident degree of hand tremor (Louis et al., 1998b). Essential tremor (ET) is a disease that is characterized by moderate to severe and often-disabling levels of tremor (Benito-Leon and Louis, 2006); it is more common than any other tremor-producing disease, and one of the most common neurological diseases, occurring in 4.0% in individuals aged ≥ 40 years and as many as 21.7% of persons aged ≥ 95 years (Benito-Leon et al., 2003; Dogu et al., 2003; Louis and Ferreira, 2009). Aside from putative genetic factors (Clark et al., 1997; Higgins et al., 1997; Shatunov et al., 2006), environmental factors (Jiménez-Jiménez FJ and B., 2007; Louis, 2001; Louis, 2008; Salemi et al., 1998) are likely to play a role in tremor etiology and two toxicants have been studied in some detail.

Harmane (1-methyl-9H-pyrido[3,4- β]indole) is a potent β -carboline alkaloid that produces tremor in laboratory animals (McKenna, 1996). Although harmane is produced endogenously, it is also present in the human diet (esp. in animal protein); this exogenous exposure is thought to be the primary source of the body burden of harmane (Pfau and Skog, 2004). Human volunteers who have been exposed to high doses of β -carboline alkaloids display a coarse action tremor (Lewin, 1928). In an environmental epidemiological study in New York, higher blood harmane concentrations were observed in ET cases than controls in two independent samples (Louis et al., 2008; Louis et al., 2002).

Lead, another neurotoxicant, has been noted to produce a variety of neurological effects in laboratory animals; among these is tremor. Laboratory animals and humans exposed to high levels of organic lead compounds develop prominent action tremor (Booze et al., 1983; Coulehan et al., 1983; Goldings and Stewart, 1982; Seshia et al., 1978; Valpey et al., 1978; Young et al., 1977). In epidemiological studies in New York (Louis et al., 2003) and Mersin Turkey (Dogu et al., 2007), blood lead (BPb) levels were elevated in ET cases compared to controls.

To date, the effects of harmane and lead with respect to tremor have been studied separately, and there has been no attempt to assess the combined effects of these two toxicants within

the same sample of individuals. To investigate this issue, we examined data on blood concentrations of both harmane and lead in a defined sample of approximately 250 individuals. These individuals, who manifested a full spectrum of tremor severity, from none to severe, were comprised of normal controls and ET cases all enrolled in an epidemiological study in New York. We tested the hypothesis that tremor would be particularly severe among individuals who had higher blood levels of both of these toxicants.

2. Methods

2.1 Participants

All participants were enrolled between December 2000 and May 2004 in a study of the environmental epidemiology of tremor at Columbia-University Medical Center (CUMC). Participants were comprised of ET cases and controls who did not have ET. By design, ET cases were identified from several sources; the major ones were a computerized billing database of patients at the Neurological Institute of New York, CUMC and the International Essential Tremor Foundation, whose members were mailed advertisements (Louis et al., 2008; Louis et al., 2002). All cases had received a diagnosis of ET from their treating neurologist and lived within two hours driving distance of CUMC in New York, New Jersey, and Connecticut. Based on a videotaped tremor examination, described below, their diagnoses were confirmed by a senior movement disorder neurologist (E.D.L.) using published diagnostic criteria (moderate or greater amplitude action tremor during three or more activities or a head tremor in the absence of Parkinson's disease, dystonia or another neurological disorder) (Louis et al., 1998a; Louis et al., 1997; Louis et al., 2002).

Normal control subjects were also recruited during the same time period (Louis et al., 2008; Louis et al., 2002). These controls were identified using random digit telephone dialing within a defined set of telephone area codes in the New York Metropolitan area that were represented by the ET cases. Controls were frequency-matched to cases based on gender, race, and current age.

The CUMC Internal Review Board approved of all study procedures, and signed written informed consent was obtained from all participants at the time of enrollment (Louis et al., 2008; Louis et al., 2002).

Of 314 ET cases and controls enrolled, data on blood harmane and BPb concentrations were available in 257 (81.9%). The majority of the remaining 57 participants had refused phlebotomy or had had an unsuccessful phlebotomy attempt. The final sample of 257 was similar to the base sample of 314 in terms of age (65.8 ± 13.8 vs. 67.0 ± 13.6 years, $t = 1.04$, $p = 0.30$), gender (146 [56.8%] vs. 179 [57.0%] female, chi-square = 0.00, $p = 0.96$), years of education (15.1 ± 3.9 vs. 15.0 ± 3.8 years, $t = 0.31$, $p = 0.76$), cigarette pack-years (9.4 ± 20.4 vs. 9.3 ± 20.3 years, $t = 0.06$, $p = 0.95$) and tremor severity (total tremor score = 10.7 ± 9.7 vs. 10.1 ± 9.5 , $t = 0.74$, $p = 0.46$).

2.2 Clinical Evaluation

All participants were evaluated in person by a trained tester. The tester administered clinical questionnaires and performed a videotaped tremor examination and phlebotomy.

Most evaluations were home visits and, therefore, were performed in the late morning or early afternoon, making fasting blood harmane concentrations impractical. Published data from other groups suggest that plasma concentrations of harmane do not change significantly during the course of the day (Rommelspacher et al., 1991). In one study (Rommelspacher et al., 1991), human subjects ingested food or ethanol, and plasma

harmane concentrations were measured hourly for eight hours. The concentration remained stable. The same investigators also demonstrated that variability in concentration was minimal over a longer (three week) period (Rommelspacher et al., 1991).

The tester used a structured questionnaire to collect demographic information including age in years, gender, years of education, race, number of rooms in home (a socioeconomic variable), current smoking status and cigarette pack-years. Medical co-morbidity was assessed with the Cumulative Illness Rating Scale, which assessed illnesses in 14 bodily systems, including cardiac, vascular, respiratory, eyes/ears/nose and throat, upper and lower gastrointestinal tracts, hepatic, renal, genitourinary, musculoskeletal, neurological, hypertension, endocrine and malignancy (range = 0 – 42 [high co-morbidity]) (Linn et al., 1968). Data on current diet were collected using a Willett Semi-Quantitative Food-Frequency Questionnaire (Willett et al., 1985). This questionnaire (Willett et al., 1985) included questions on frequency of current consumption of 61 foods and on the use of vitamins and mineral supplements. Food frequency data were used to compute mean daily intake of alcohol (grams), calories (kilocalories) and animal protein consumption.

The tester videotaped a tremor examination in all participants (Louis et al., 1997; Louis et al., 2002). Each of 12 videotaped action tremor items (arm extension, pouring, drinking, use a spoon, finger-nose-finger maneuver, and writing with each arm) was rated by a senior movement disorder neurologist (E.D.L.) on a scale from 0 (none) to 3 (severe tremor), and a total tremor score was assigned (range = 0 – 36) (Louis et al., 1998a; Louis et al., 1997; Louis et al., 2002).

2.3 Blood Harmane Concentrations

At the time of the evaluation, phlebotomy was performed. Blood harmane concentrations were measured blinded to all clinical information with a well-established high performance liquid chromatography method described in detail in our previous studies (Louis et al., 2008; Louis et al., 2002; Zheng et al., 2000). The intraday precision, measured as a coefficient of variation at 25 ng/mL, was 6.7%. The interday precision was 7.3% (Zheng et al., 2000).

2.4 BPb Concentrations

As described previously (Louis et al., 2003), venous blood samples were collected in lead-free tubes and then analyzed using graphite furnace atomic absorption spectrophotometry (Perkin-Elmer Analyst 600; Perkin Elmer, Chelmsford, MA) in the NIEHS Trace Metal Laboratory at Columbia University. These analyses were performed blinded to all clinical information. The detection limit for BPb measurements using these instruments was 0.1 ug/dl. Day-to-day variability was 3.7%. The laboratory participates in the BPb lead quality control program of the Centers for Disease Control. The intraclass correlation coefficient, which quantifies the association between the measured and the quality control values for BPb, was 0.99 during the course of this study.

2.5 Statistical Analyses

Statistical analyses were performed in SPSS (Version 17.0). The empirical distribution of blood harmane concentration, BPb concentration and total tremor score were positively skewed (respective one-sample Kolmogorov-Smirnov tests, $z = 6.59, 2.05, \text{ and } 3.01$ [all $p < 0.001$]). Hence, non-parametric tests (Mann Whitney U, Kruskal-Wallis, Spearman's rho) were used when assessing these variables.

Blood harmane concentration was divided into a high vs. low group based on the median value. The same was done for BPb concentration. Study participants were then stratified into four toxicant groups: (Group 1) low blood harmane and BPb concentrations, (Group 2) low

blood harmaline and high BPb concentration, (Group 3) high blood harmaline and low BPb concentration, and (Group 4) high blood harmaline and BPb concentrations. Total tremor score was compared across groups. In a linear regression model in which log-total tremor score was the dependent variable, toxicant group (Group 1 [both low], Groups 2+3 [either high], Group 4 [both high]) was the independent variable, we examined the association between these toxicant groups and total tremor score. In multivariate linear regression models, we adjusted for variables that were associated at the $p < 0.1$ level with either blood harmaline concentration, BPb concentration or total tremor score in univariate analyses. We did not adjust for dietary variables (e.g., daily intakes of alcohol, calories or animal protein) because these variables are antecedent variables rather than confounders (e.g., increased animal protein consumption could lead to increased blood harmaline concentration that then leads to tremor).

Because the total tremor score was a continuous, quantitative measure, we combined cases and controls for our main analyses. In a set of secondary analyses, we also examined data on cases and controls separately.

3. Results

There were 257 participants (151 controls and 106 ET cases, age range = 21 – 91 years) who had both blood harmaline and BPb concentrations (Table 1). The total tremor scores ranged from 0 – 36, and with only one exception (total tremor score = 28), all values between 0 and 36 were represented. Thus, a full spectrum of tremor severities was represented by these participants. As expected, the mean total tremor score was higher in ET cases than controls (20.1 ± 7.8 vs. 3.9 ± 2.8 , Mann Whitney $z = 13.0$, $p < 0.001$).

Blood harmaline and lead concentrations are shown (Table 1). We examined the correlates of blood harmaline concentration, BPb concentration, and tremor severity (total tremor score) (Table 2). Each of these increased with age (Table 2). Both BPb concentration and total tremor score differed by gender, with men having higher BPb concentrations and higher total tremor scores. White race and cigarette pack-years covaried with total tremor score (Table 2). Several other correlations were noted, including an association between daily animal protein consumption and blood harmaline concentrations (Table 2). There was no correlation between blood harmaline and BPb concentrations (Spearman's $r = 0.008$, $p = 0.90$).

Blood harmaline concentration correlated with total tremor score (Spearman's $r = 0.17$, $p = 0.007$); BPb concentration also correlated with total tremor score (Spearman's $r = 0.13$, $p = 0.045$). Figure 1 depicts the log total tremor score by log blood harmaline concentration, both in participants with low BPb concentration and in participants with high BPb concentration. Tremor was higher in participants with high BPb concentration than in participants with low BPb concentration. Also, tremor increased with increasing blood harmaline concentration, but the increase was not modified by BPb concentration (i.e., there was no evidence of effect modification, Figure 1).

Blood harmaline concentration was divided into a high vs. low group based on the median value. The same was done for BPb concentration. Study participants were then stratified into four toxicant groups: (Group 1) low blood harmaline and BPb concentrations, (Group 2) low blood harmaline and high BPb concentration, (Group 3) high blood harmaline and low BPb concentration, and (Group 4) high blood harmaline and BPb concentrations. The total tremor score was lowest in Group 1, intermediate in Groups 2 and 3, and highest in Group 4 (Kruskal-Wallis $p = 0.028$, Table 3). Collapsing Groups 2 and 3 into a single group (each

had only one high toxicant concentration) produced similar results (Kruskal-Wallis $p = 0.01$, Table 3).

In a linear regression model in which log-total tremor score was the dependent variable and toxicant group (1, 2+3, 4) was the independent variable, we found a significant association ($\beta = 0.116$, $p = 0.006$). Age was the major confounding factor, and in a model that adjusted for age in years, the association between toxicant group and total tremor score persisted ($\beta = 0.098$, $p = 0.02$). In a model that adjusted for age in years and then further adjusted for gender, white race and cigarette pack-years (each associated at the $p < 0.1$ level with either blood harmane concentration, BPb concentration or total tremor score in univariate analyses, Table 2), the association between toxicant group and total tremor score persisted ($\beta = 0.087$, $p = 0.04$).

In a secondary analysis, restricted to the 106 ET cases, results were similar. Total tremor score was associated with toxicant group: (Group 1 = 17.3 ± 5.5 , Group 2 + 3 = 20.1 ± 8.1 , and Group 4 = 22.3 ± 7.1 , Kruskal-Wallis $p = 0.08$) and, in linear regression models, there was an association between toxicant group and total tremor score (unadjusted model, $\beta = 0.06$, $p = 0.01$; model adjusting for age in years, gender, white race and cigarette pack-years, $\beta = 0.06$, $p = 0.018$). In another secondary analysis, restricted to the 151 controls, total tremor score rose with toxicant group, but not to a significant degree: (Group 1 = 3.5 ± 2.3 , Group 2 + 3 = 3.9 ± 2.9 , and Group 4 = 4.8 ± 3.1 , Kruskal-Wallis $p = 0.24$), although these analyses were limited by the restricted range of tremor scores seen in controls. In linear regression models (analyses restricted to controls), there was no association between toxicant group and total tremor score (unadjusted model, $\beta = 0.06$, $p = 0.16$; model adjusting for age in years, gender, white race and cigarette pack-years, $\beta = 0.05$, $p = 0.26$).

4. Discussion

Environmental factors are likely to play a role in tremor etiology and two toxicants, in particular, have been studied in some detail. Yet the relationships between blood harmane and tremor and between BPb and tremor have each been studied separately. There has been no attempt to assess the relationship between these toxicants and tremor within the same group of individuals. We collected data on blood concentrations of both toxicants in more than 250 individuals who represented a full spectrum of tremor severity, from none to severe. We hypothesized that tremor would be particularly severe among individuals who had higher blood levels of both of these toxicants. As hypothesized, the blood harmane concentration correlated with total tremor score, as did the BPb concentration. Participants who had high levels of both toxicants had the highest tremor scores, demonstrating what could be an additive effect of the two toxicants on tremor severity.

As noted above, our findings are consistent with the hypothesis that there is an additive effect of the two toxicants on tremor severity. However, other possibilities should also be considered. First, there is the possibility that the observed association is the result of confounding. Prior analyses in this epidemiological study of the effects of other toxins on tremor, including manganese, organic solvents, and pesticides (Louis, 2008; Louis et al., 2004; Louis et al., 2006), suggest that these toxins did not confound these analyses. More specifically, we administered detailed occupational histories to nearly 400 ET cases and controls and these were reviewed by an industrial hygienist. In a series of papers, we did not find any associations between tremor and manganese or solvent exposures (Louis et al., 2004) or between tremor and pesticide exposure (Louis et al., 2006). Based on these null findings, we did not regard these neurotoxicants as confounders in the current analyses. Nevertheless, we recognize that although we took a large number of important variables into

consideration, we realize that residual confounding from other unmeasured factors could conceivably be present. Second, there is the possibility of indirect association. We think this is unlikely because of the wealth of animal experimental data showing that administration of harmane and other β -carboline alkaloids results in acute action tremor (Fuentes and Longo, 1971; Martin et al., 2005; Robertson, 1980). Furthermore, human volunteers exposed under experimental conditions to high doses of β -carboline alkaloids display a coarse action tremor (Lewin, 1928).

β -carboline alkaloids such as harmane are highly neurotoxic, and their administration to a wide variety of laboratory animals produces action tremor. Aside from harmane, the β -carboline alkaloids include harmine, harmaline, and other compounds with a similar chemical structure. Although β -carboline alkaloids are produced endogenously in the human body (Gearhart et al., 2000; Wakabayashi et al., 1997), harmane is also a dietary neurotoxin, and dietary sources are estimated to be far greater than endogenous sources (Pfau and Skog, 2004). Humans may be exposed to low levels of harmane through a variety of foods, including vegetable-derived and animal derived, with concentrations in animal protein considered to be among the highest. Harmane and other β -carboline alkaloids are found in particularly high ng/gm concentrations in muscle foods (beef, chicken and pork) and cooking leads to furthermore increased concentrations (Louis, 2008). Indeed, the formation of β -carboline alkaloids in cooked meat is a function of cooking temperature and time, with β -carboline alkaloid concentrations increasing most rapidly with time at higher temperatures (Louis, 2008). Pan frying and grill/barbequing produce the highest concentrations of β -carboline alkaloids (Louis, 2008). The mechanism whereby these toxicants produce tremor is not fully known, but likely involves the physical disruption of cerebellar input fibers and Purkinje cell populations in the cerebellum (Du et al., 1997; Milner et al., 1995; O'Hearn et al., 1993; O'Hearn and Molliver, 1993; O'Hearn and Molliver, 1997; Robertson, 1980; Sinton et al., 1989), an area of the brain recently shown to be characterized by degenerative changes in patients with tremor disorders such as ET (Louis et al., 2007a). There are few other data on the expected blood concentrations of harmane in the population. A study by Kuhn et al (Kuhn et al., 1995) in Germany measured harmane in plasma samples of 36 control subjects who were patients with cardiovascular disorders, and values were $0.04 \pm 0.09 \text{ g}^{-10}/\text{ml}$ (range = 0 - $0.51 \text{ g}^{-10}/\text{ml}$) compared to $0.15 \pm 0.72 \text{ g}^{-10}/\text{ml}$ (range = $0.00037 - 8.68 \text{ g}^{-10}/\text{ml}$) in controls in the present study. While the ranges overlap, our mean value appears to be slightly higher and this is likely to be a function of differences between the two methodologies. Our method uses whole blood (Zheng et al., 2000) whereas the German study (Kuhn et al., 1995) only used plasma. Harmane is highly lipophilic, accumulating inside of blood cells, with published studies demonstrating low relative recovery of harmane from plasma (Guan et al., 2001; Zheng et al., 2000).

Lead, another tremor-producing neurotoxicant (Booze et al., 1983; Coulehan et al., 1983; Goldings and Stewart, 1982; Seshia et al., 1978; Valpey et al., 1978; Young et al., 1977), is ubiquitous (Schroeder and Tipton, 1968) and humans may be exposed to both inorganic and organic forms of lead from occupational and non-occupational sources (Coulehan et al., 1983; Winegar et al., 1977). Destruction of cerebellar Purkinje cells is a major feature of the pathology of the toxicity of organic lead compounds in humans (Valpey et al., 1978).

We show here that presence of higher levels of both toxicants, together in the same individual, is associated with more tremor. It is not known whether the biological mechanism whereby this occurs is an additive set of physiological or pathological changes in Purkinje cells or other cerebellar neuronal populations. This requires further exploration.

We observed that BPb concentrations were higher in men than women. A gender difference, with males having higher blood lead levels than females, has been reported in numerous

other studies both in the United States and elsewhere (Counter et al., 2001; Harlan, 1988; Kurtin et al., 1997; Moralez et al., 2005; Nriagu et al., 2006). Several possible explanations have been proposed, including increased skeletal mass in men resulting in increased body stores of lead and the higher concentration of red blood cells in men (a large percentage of lead is bound to these cells) (Nriagu et al., 2006).

This study had limitations. First, as in our prior studies (Louis et al., 2008; Louis et al., 2005; Louis et al., 2002; Louis et al., 2007b), we did not assess fasting blood harmaline concentrations because it was impractical to do so. This raises the possibility that the blood harmaline concentration merely reflected immediate dietary influences. However, our prior data indicate that blood harmaline concentration is not correlated with the time latency since last food consumption ($r = -0.097$, $p = 0.49$, in 52 participants) (Louis et al., 2008). Published data from other groups suggest that plasma concentrations of harmaline do not change significantly during the course of the day (Rommelspacher et al., 1991). Second, we realize that these results, which were derived from a study with a modest sample size, should be regarded as preliminary and they will need to be replicated in other studies. Our study sample was predominantly Caucasian and well-educated and the ability to generalize these results to other populations is an issue that must be addressed in future studies. Third, while we demonstrated an association between these toxins and tremor, this was a cross-sectional study, and we were not able to show that these exposures resulted in tremor. Fourth, we did not assess anxiety or depression or their role as potential confounders. The study also had several strengths. First, both blood harmaline and BPb concentrations were assessed in the same study sample, comprising a collection of more than 250 individuals. Second, given the presence of normal controls as well as individuals with ET, a full spectrum of tremor severity was present. Finally, there is the uniqueness of the question; there are no other studies that have examined this issue.

In summary, we show here that blood harmaline concentration correlated with total tremor score, as did BPb concentration. Participants who had high levels of both toxicants had the highest tremor scores, demonstrating what may have been an additive effect of the two toxicants on tremor severity. The study of environmental factors that contribute to tremor is in its infancy. Given the very high prevalence of tremor disorders in the population (Benito-Leon et al., 2003), as well as the possibility that such tremors may be severe and functionally disabling (Louis et al., 2001), identifying environmental determinants is important in terms of primary disease prevention.

References

- Benito-Leon J, Bermejo-Pareja F, Morales JM, Vega S, Molina JA. Prevalence of essential tremor in three elderly populations of central Spain. *Mov Disord.* 2003; 18:389–394. [PubMed: 12671944]
- Benito-Leon J, Louis ED. Essential tremor: emerging views of a common disorder. *Nat Clin Pract Neurol.* 2006; 2:666–678. [PubMed: 17117170]
- Booze RM, Mactutus CF, Annau Z, Tilson HA. Neonatal triethyl lead neurotoxicity in rat pups: initial behavioral observations and quantification. *Neurobehav Toxicol Teratol.* 1983; 5:367–375. [PubMed: 6877478]
- Clark LN, Park N, Kisselev S, Rios E, Lee JH, Louis ED. Replication of the LINGO1 gene association with essential tremor in a North American population. *Eur J Hum Genet.* 2010; 18:838–843. [PubMed: 20372186]
- Coulehan JL, Hirsch W, Brillman J, Sanandria J, Welty TK, Colaiaco P, et al. Gasoline sniffing and lead toxicity in Navajo adolescents. *Pediatrics.* 1983; 71:113–117. [PubMed: 6848958]
- Counter SA, Buchanan LH, Ortega F. Gender differences in blood lead and hemoglobin levels in Andean adults with chronic lead exposure. *Int J Occup Environ Health.* 2001; 7:113–118. [PubMed: 11373041]

- Dogu O, Louis ED, Tamer L, Unal O, Yilmaz A, Kaleagasi H. Elevated blood lead concentrations in essential tremor: a case-control study in Mersin, Turkey. *Environ Health Perspect.* 2007; 115:1564–1568. [PubMed: 18007985]
- Dogu O, Sevim S, Camdeviren H, Sasmaz T, Bugdayci R, Aral M, et al. Prevalence of essential tremor: door-to-door neurologic exams in Mersin Province, Turkey. *Neurology.* 2003; 61:1804–1806. [PubMed: 14694055]
- 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. [PubMed: 9372198]
- Fuentes JA, Longo VG. An investigation on the central effects of harmine, harmaline and related beta-carbolines. *Neuropharmacology.* 1971; 10:15–23. [PubMed: 5569304]
- Gearhart DA, Collins MA, Lee JM, Neafsey EJ. Increased beta-carboline 9N-methyltransferase activity in the frontal cortex in Parkinson's disease. *Neurobiol Dis.* 2000; 7:201–211. [PubMed: 10860785]
- Goldings AS, Stewart RM. Organic lead encephalopathy: behavioral change and movement disorder following gasoline inhalation. *J Clin Psychiatry.* 1982; 43:70–72. [PubMed: 7056707]
- Guan Y, Louis ED, Zheng W. Toxicokinetics of tremorogenic natural products, harmaline and harmine, in male Sprague-Dawley rats. *J Toxicol Environ Health A.* 2001; 64:645–660. [PubMed: 11766171]
- Gulcher JR, Jonsson P, Kong A, Kristjansson K, Frigge ML, Karason A, et al. Mapping of a familial essential tremor gene, FET1, to chromosome 3q13. *Nat Genet.* 1997; 17:84–87. [PubMed: 9288103]
- Harlan WR. The relationship of blood lead levels to blood pressure in the U.S. population. *Environ Health Perspect.* 1988; 78:9–13. [PubMed: 3203651]
- Higgins JJ, Pho LT, Nee LE. A gene (ETM) for essential tremor maps to chromosome 2p22–p25. *Mov Disord.* 1997; 12:859–864. [PubMed: 9399207]
- Jiménez-Jiménez, FJdT-HM.; Alonso-Navarro, H.; Ayuso-Peralta, L.; Arévalo-Serrano, J.; Ballesteros-Barranco, A.; Puertas, I.; Jabbour-Wadih, T.; Barcenilla, B. Environmental risk factors for essential tremor. *Eur Neurol.* 2007; 58:106–113. [PubMed: 17570916]
- Kuhn W, Muller T, Grosse H, Rommelspacher H. Plasma harman and norharman in Parkinson's disease. *J Neural Transm Suppl.* 1995; 46:291–295. [PubMed: 8821066]
- Kurtin D, Therrell BL Jr, Patterson P. Demographic risk factors associated with elevated lead levels in Texas children covered by Medicaid. *Environ Health Perspect.* 1997; 105:66–68. [PubMed: 9074883]
- Lewin L. Untersuchungen Uber Banisteria caapi Sp. *Arch Exp Pathol Pharmacol.* 1928; 129:133–149.
- Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. *J Am Geriatr Soc.* 1968; 16:622–626. [PubMed: 5646906]
- Louis ED. Etiology of essential tremor: should we be searching for environmental causes? *Mov Disord.* 2001; 16:822–829. [PubMed: 11746611]
- Louis ED. Environmental Epidemiology of Essential Tremor. *Neuroepidemiology.* 2008; 31:139–149. [PubMed: 18716411]
- Louis ED, Applegate LM, Factor-Litvak P, Parides MK, Andrews L. Essential tremor: occupational exposures to manganese and organic solvents. *Neurology.* 2004; 63:2162–2164. [PubMed: 15596771]
- Louis ED, Barnes L, Albert SM, Cote L, Schneier FR, Pullman SL, et al. Correlates of functional disability in essential tremor. *Mov Disord.* 2001; 16:914–920. [PubMed: 11746622]
- Louis ED, Factor-Litvak P, Parides M, Andrews L, Santella RM, Wolff MS. Organochlorine pesticide exposure in essential tremor: a case-control study using biological and occupational exposure assessments. *Neurotoxicology.* 2006; 27:579–586. [PubMed: 16620996]
- Louis ED, Faust PL, Vonsattel JP, Honig LS, Rajput A, Robinson CA, et al. Neuropathological changes in essential tremor: 33 cases compared with 21 controls. *Brain.* 2007a; 130:3297–3307. [PubMed: 18025031]
- Louis ED, Ferreira JJ. How common is the most common adult movement disorder? Update on the worldwide prevalence of essential tremor. *Mov Disord.* 2010; 25:534–541. [PubMed: 20175185]

- Louis ED, Ford B, Lee H, Andrews H. Does a screening questionnaire for essential tremor agree with the physician's examination? *Neurology*. 1998a; 50:1351–1357. [PubMed: 9595986]
- Louis ED, Ford B, Pullman S, Baron K. How normal is 'normal'? Mild tremor in a multiethnic cohort of normal subjects. *Arch Neurol*. 1998b; 55:222–227. [PubMed: 9482364]
- Louis ED, Jiang W, Pellegrino KM, Rios E, Factor-Litvak P, Henchcliffe C, et al. Elevated blood harmaline (1-methyl-9H-pyrido[3,4-b]indole) concentrations in essential tremor. *Neurotoxicology*. 2008; 29:294–300. [PubMed: 18242711]
- Louis ED, Jurewicz EC, Applegate L, Factor-Litvak P, Parides M, Andrews L, et al. Association between essential tremor and blood lead concentration. *Environ Health Perspect*. 2003; 111:1707–1711. [PubMed: 14594619]
- Louis ED, Ottman R, Ford B, Pullman S, Martinez M, Fahn S, et al. The Washington Heights-Inwood Genetic Study of Essential Tremor: methodologic issues in essential-tremor research. *Neuroepidemiology*. 1997; 16:124–133. [PubMed: 9159767]
- Louis ED, Thawani SP, Andrews HF. Prevalence of essential tremor in a multiethnic, community-based study in northern Manhattan, New York, N.Y. *Neuroepidemiology*. 2009; 32:208–214. [PubMed: 19169043]
- Louis ED, Zheng W, Applegate L, Shi L, Factor-Litvak P. Blood harmaline concentrations and dietary protein consumption in essential tremor. *Neurology*. 2005; 65:391–396. [PubMed: 16087903]
- Louis ED, Zheng W, Jurewicz EC, Watner D, Chen J, Factor-Litvak P, et al. Elevation of blood beta-carboline alkaloids in essential tremor. *Neurology*. 2002; 59:1940–1944. [PubMed: 12499487]
- Louis ED, Zheng W, Mao X, Shungu DC. Blood harmaline is correlated with cerebellar metabolism in essential tremor: a pilot study. *Neurology*. 2007b; 69:515–520. [PubMed: 17679670]
- Martin FC, Thu Le A, Handforth A. Harmaline-induced tremor as a potential preclinical screening method for essential tremor medications. *Mov Disord*. 2005; 20:298–305. [PubMed: 15580562]
- McKenna DJ. Plant hallucinogens: springboards for psychotherapeutic drug discovery. *Behav Brain Res*. 1996; 73:109–116. [PubMed: 8788486]
- 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. [PubMed: 7666163]
- Morales LS, Gutierrez P, Escarce JJ. Demographic and socioeconomic factors associated with blood lead levels among Mexican-American children and adolescents in the United States. *Public Health Rep*. 2005; 120:448–454. [PubMed: 16025725]
- Nriagu J, Burt B, Linder L, Ismail A, Sohn W. Lead levels in blood and saliva in a low-income population of Detroit, Michigan. *Int J Hyg Environ Health*. 2006; 209:109–121. [PubMed: 16443391]
- O'Hearn E, Long DB, Molliver ME. Ibogaine induces glial activation in parasagittal zones of the cerebellum. *Neuroreport*. 1993; 4:299–302. [PubMed: 8477052]
- 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. [PubMed: 8377927]
- O'Hearn E, Molliver ME. The olivocerebellar projection mediates ibogaine-induced degeneration of Purkinje cells: a model of indirect, trans-synaptic excitotoxicity. *J Neurosci*. 1997; 17:8828–8841. [PubMed: 9348351]
- Pfau W, Skog K. Exposure to beta-carbolines norharman and harman. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2004; 802:115–26.
- Robertson HA. Harmaline-induced tremor: the benzodiazepine receptor as a site of action. *Eur J Pharmacol*. 1980; 67:129–132. [PubMed: 6252023]
- Rommelspacher H, Schmidt LG, May R. Plasma norharman (beta-carboline) levels are elevated in chronic alcoholics. *Alcohol Clin Exp Res*. 1991; 15:553–559. [PubMed: 1877743]
- Salemi G, Aridon P, Calagna G, Monte M, Savettieri G. Population-based case-control study of essential tremor. *Ital J Neurol Sci*. 1998; 19:301–305. [PubMed: 10933450]
- Schroeder HA, Tipton IH. The human body burden of lead. *Arch Environ Health*. 1968; 17:965–978. [PubMed: 4177349]

- Seshia SS, Rjani KR, Boeckx RL, Chow PN. The neurological manifestations of chronic inhalation of leaded gasoline. *Dev Med Child Neurol.* 1978; 20:323–334. [PubMed: 97111]
- Shatunov A, Sambuughin N, Jankovic J, Elble R, Lee HS, Singleton AB, et al. Genomewide scans in North American families reveal genetic linkage of essential tremor to a region on chromosome 6p23. *Brain.* 2006; 129:2318–2331. [PubMed: 16702189]
- Sinton CM, Krosser BI, Walton KD, Llina RR. The effectiveness of different isomers of octanol as blockers of harmaline-induced tremor. *Pflugers Arch.* 1989; 414:31–36. [PubMed: 2542888]
- Valpey R, Sumi SM, Copass MK, Goble GJ. Acute and chronic progressive encephalopathy due to gasoline sniffing. *Neurology.* 1978; 28:507–510. [PubMed: 565491]
- 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. [PubMed: 9202762]
- Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol.* 1985; 122:51–65. [PubMed: 4014201]
- Winegar DA, Levy BS, Andrews JS Jr, Landrigan PJ, Scruton WH, Krause MJ. Chronic occupational exposure to lead: an evaluation of the health of smelter workers. *J Occup Med.* 1977; 19:603–606. [PubMed: 599390]
- Young RS, Grzyb SE, Crismon L. Recurrent cerebellar dysfunction as related to chronic gasoline sniffing in an adolescent girl. Lead poisoning from “leaded” gasoline as an attendant complication. *Clin Pediatr (Phila).* 1977; 16:706–708. [PubMed: 872480]
- Zheng W, Wang S, Barnes LF, Guan Y, Louis ED. Determination of harmaline and harmine in human blood using reversed-phased high-performance liquid chromatography and fluorescence detection. *Anal Biochem.* 2000; 279:125–129. [PubMed: 10706780]

Figure 1.

Log total tremor score (Y axis) by log blood harmane concentration (X axis). Participants with low BPb concentration are indicated by open squares and their data are reflected by the bottom (dashed) fit line. Participants with high BPb concentration are indicated by closed circles and their data are reflected by the top (solid) fit line. Occasional closed squares represent the overlap of closed circles and open squares.

Table 1**Demographic and Clinical Characteristics of 257 Participants**

	All	ET	Controls
N	257	106	151
Age (years)	65.8 ± 13.8	68.2 ± 15.2	64.1 ± 12.5
Female gender	146 (56.8%)	59 (55.7%)	87 (57.6%)
White Race	235 (91.4%)	100 (94.3%)	135 (89.4%)
Education (years)	15.1 ± 3.9	14.6 ± 4.3	15.4 ± 3.5
Number of rooms in home	5.7 ± 2.4	5.8 ± 2.6	5.7 ± 2.3
Current smoker	23 (8.9%)	9 (8.5%)	14 (9.3%)
Cigarette pack-years ^a	9.4 ± 20.4	8.2 ± 19.8	10.2 ± 20.8
CIRS score	4.9 ± 3.6	5.1 ± 3.5	4.7 ± 3.7
Total Tremor Score	10.7 ± 9.7	20.1 ± 7.8	3.9 ± 2.8
Daily alcohol consumption (grams)	7.2 ± 11.5	8.6 ± 13.2	6.2 ± 10.0
Daily caloric consumption (Kcal)	1439 ± 451	1426 ± 386	1448 ± 492
Daily animal protein consumption (grams)	50.3 ± 19.3	50.7 ± 19.3	50.1 ± 19.4
Blood harmane concentration in g ⁻¹⁰ /ml (median, range)	0.17 ± 0.74 (0.02, 0.00037 – 8.68)	0.20 ± 0.77 (0.027, 0.00037 – 7.56)	0.15 ± 0.72 (0.027, 0.00037 – 8.68)
BPb concentration in ug/dl (median, range)	2.9 ± 1.8 (2.5, 0.3 – 11.9)	3.1 ± 2.1 (2.7, 0.3 – 11.6)	2.7 ± 1.6 (2.4, 0.3 – 11.9)

Values are means ± standard deviation or number followed by (percent).

^aNon-smokers were assigned a value of 0.

CIRS = Cumulative Illness Rating Scale score

Table 2

Correlates of Blood Harmane Concentration, BPb Concentration, and Tremor Severity

	Blood Harmane Concentration	BPb Concentration	Total Tremor Score
Age (years) ^a	$r = 0.14, p = 0.025$	$r = 0.13, p = 0.04$	$r = 0.24, p < 0.001$
Gender ^b			
Men	$0.20 \pm 0.85 (0.02)$	$3.29 \pm 1.61 (3.0)$	$11.4 \pm 9.0 (8.0)$
Women	$0.15 \pm 0.65 (0.02)$	$2.57 \pm 1.89 (2.1)$	$10.1 \pm 10.2 (5.0)$
	$p = 0.96$	$p < 0.001$	$p = 0.03$
White Race ^b			
Yes	$0.09 \pm 0.21 (0.02)$	$2.96 \pm 1.43 (2.7)$	$6.6 \pm 6.3 (5.0)$
No	$0.18 \pm 0.79 (0.02)$	$2.84 \pm 1.83 (2.4)$	$11.1 \pm 9.9 (7.0)$
	$p = 0.53$	$p = 0.35$	$p = 0.07$
Years of Education ^a	$r = -0.06, p = 0.32$	$r = -0.007, p = 0.91$	$r = -0.08, p = 0.19$
Number of Rooms in Home ^a	$r = 0.02, p = 0.76$	$r = 0.06, p = 0.36$	$r = -0.01, p = 0.90$
Current smoker ^b			
Yes	$0.18 \pm 0.79 (0.02)$	$2.82 \pm 1.81 (2.46)$	$10.6 \pm 9.8 (6.0)$
No	$0.06 \pm 0.11 (0.02)$	$3.15 \pm 1.65 (2.59)$	$11.4 \pm 9.1 (7.5)$
	$p = 0.49$	$p = 0.17$	$p = 0.35$
Cigarette Pack-Years ^a	$r = -0.09, p = 0.13$	$r = -0.06, p = 0.33$	$r = -0.13, p = 0.04$
Cumulative Illness Rating Scale score ^a	$r = 0.005, p = 0.93$	$r = -0.097, p = 0.12$	$r = 0.058, p = 0.36$
Daily alcohol consumption (grams)	$r = -0.08, p = 0.23$	$r = 0.22, p = 0.001$	$r = 0.005, p = 0.94$
Daily caloric consumption (kilocalories)	$r = 0.10, p = 0.11$	$r = 0.02, p = 0.71$	$r = -0.01, p = 0.86$
Daily animal protein consumption (grams)	$r = 0.13, p = 0.045$	$r = -0.04, p = 0.58$	$r = -0.02, p = 0.79$

^aSpearman's rho was used for statistical comparisons.^bValues are mean $\pm$ S.D. (median). Mann Whitney U was used for statistical comparisons.

Table 3**Total Tremor Score by Toxicant Group**

Toxicant Group	Total Tremor Score
Group 1 (↓harmaline and ↓lead)	8.4 ± 8.2 (5)
Group 2 (↑harmaline and ↓lead)	10.8 ± 10.0 (6)
Group 3 (↓harmaline and ↑lead)	10.1 ± 9.6 (6)
Group 4 (↑harmaline and ↑lead)	13.7 ± 10.4 (12)
Kruskal-Wallis p = 0.028	
Group 1 (↓harmaline and ↓lead)	8.4 ± 8.2 (5)
Group 2 + Group 3	10.5 ± 9.8 (6)
Group 4 (↑harmaline and ↑lead)	13.7 ± 10.4 (12)
Kruskal-Wallis p = 0.01	

Values are means ± standard deviation (median).

↓harmaline < 0.02 g⁻¹⁰/ml; ↑harmaline ≥ 0.02 g⁻¹⁰/ml.

↓BPb < 2.5 ug/dl; ↑BPb ≥ 2.5 ug/dl.