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Nicotine in the hair of mummies from San Pedro de Atacama (Northern Chile)

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ABSTRACT

The consumption of plant-derived hallucinogenic substances through smoking and snuffing is a long-standing tradition in the south-central Andes. Chemical and archaeobotanical evidence point to the consumption of nicotine and tryptamine alkaloids in Northwestern Argentina and of tryptamine alkaloids in San Pedro de Atacama (SPA), in prehispanic times. In this paper, results are reported of gas chromatography–mass spectrometry (GC/MS) analyses aimed at identifying nicotine and tryptamine alkaloids in the hair of mummies from different cultural periods of SPA. Fifty-six samples were examined. While tryptamines were not found in any of the samples, nicotine was found in 35 samples, assigned to the Late Formative (1 of 1 sample from this period), Late Formative or Middle (1 of 2 samples from either of these periods), Middle (4 of 6 samples from this period) and Late Intermediate periods (8 of 12 samples from this period), or without assignment to period due to lack of contextual information (21 of 35 samples unassigned to a period). These results show a continuous consumption of nicotine from the Late Formative to the Late Intermediate periods of SPA (ca. 100 B.C.–1450 A.D.). No associations were found between presence of nicotine in the hair of mummies and presence of snuffing trays or of other snuffing paraphernalia in the corresponding tomb; furthermore, neither the diversity of the funerary context, measured in terms of the number of types of objects, nor the presence of gemstone necklaces differed between tombs with mummies with or without nicotine in their hair. Overall, these results suggest that consumption of nicotine was performed by members of the society at large, irrespective of their social and wealth status.

1. Introduction

The consumption of plant-derived hallucinogenic substances through smoking and snuffing is a long-standing tradition in the south-central Andes (Torres, 1986, 1999; Torres and Repke, 2006). While smoking pipes have been found in northwestern Argentina (NWA) in archaeological sites dated as far back as 2100 B.C. (Aschero and Yacobaccio, 1994; Fernández Distel, 1980; Pérez Gollán and Gordillo, 1993, 1994), in San Pedro de Atacama (SPA) in Northern Chile they appear later and are gradually replaced in the archaeological record by snuff trays (Llagostera, 1996; Torres, 1999). Occurrence of snuff trays in SPA is concomitant with the appearance of the first elements associated to the Tiwanaku tradition (Berenguer et al., 1986); a use as vehicles for religious proselytism has been suggested for snuff trays (Berenguer, 1998).

It has been suggested that mummies found with associated snuffing paraphernalia correspond to individuals who performed shamanic activities (Llagostera et al., 1988), that snuffing implements correspond to status symbols (Berenguer and Dauelsberg, 1989; Llagostera et al., 1988), and that hallucinogen consumption was widespread in the prehispanic SPA society (Thomas et al., 1984). A possible way to distinguish between these possibilities is to verify consumption of hallucinogens in mummies and to explore possible relationships between consumption and presence of snuff trays in the funerary context and also diversity of such context.

Two main sources of hallucinogenic compounds in the south-central Andes are nicotine-containing species of *Nicotiana* (Solanaceae), commonly referred to as tobacco, and tryptamine-containing species of *Anadenanthera* (Fabaceae), commonly referred to as cebil. Thus, chemical analysis of residues in smoking pipes from NWA revealed the presence of tryptamine alkaloids (Fernández Distel, 1980; Aschero and Yacobaccio, 1994) while in other instances, archaeobotanical analyses of pipe residues showed the presence of *Nicotiana* sp. (Capparelli et al., 2006). On the other hand, chemical analysis of the snuff powder contained in leather pouches found in two funerary contexts of SPA showed the presence of several dimethyltryptamines, particularly 5-hydroxy-N,N-dimethyltryptamine (bufotenine), which was taken as evidence for

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the consumption of *Anadenanthera* sp. (Torres et al., 1991); furthermore, thin layer chromatography coupled to a positive spot test for indoles suggested the presence tryptamines in residues associated to a Middle period snuff tray found near the coastal town of Antofagasta, Chile (Gili Hanish et al., 2009). Additionally, archaeobotanical analysis of powdered material in snuffing paraphernalia from NWA showed the presence of *Anadenanthera* sp. (Pochettino et al., 1999). In view of these antecedents, the present analyses were focused on the detection and quantitation of dimethyltryptamines and nicotine.

Consumption of psychotropic substances by modern populations has been widely assessed through analysis of residues in hair (Srogi, 2006; Man et al., 2009). However, such studies have been particularly enlightening in archaeological contexts (Wilson, 2005). Chemical analysis of hair from mummies from the area around Arica in Northern Chile has shown intake of cocaine, most likely from coca leaves (Cartmell et al., 1991a,b; Springfield et al., 1993; Rivera et al., 2005), while a similar study from the same general geographic area suggested the consumption of harmine (Ogalde et al., 2009; but see Trout, 2008), an alkaloid and monoaminooxidase inhibitor (MAO-I) present in the vine *Banisteriopsis* sp. (Malpighiaceae). On the other hand, an analysis of hair of mummies from sites along the Loa valley, also in Northern Chile, showed the absence of tropane and opioid alkaloids (Báez et al., 2000).

In this paper, the results of analyses using gas chromatography mass spectrometry (GC/MS) aimed at identifying nicotine and tryptamine alkaloids in the hair of mummies from different cultural periods of SPA are reported. The relationships between presence or absence of nicotine in mummy's hair with presence or absence of snuffing paraphernalia in the funerary context and the diversity of such context, are also reported.

2. Materials and methods

2.1. Anthropological samples

All mummies housed at the Instituto de Investigaciones y Museo R.P. Gustavo Le Paige de San Pedro de Atacama were examined for the presence of hair. Many of them were wrapped in a complex funerary bundle and their hair was not accessible. Hair samples (one sample from each individual) could be withdrawn from 56 of the ca. 450 existing mummies (Table 1). In addition, one adult hair sample of a current consumer of cebil and tobacco was analyzed as a positive control, and ten contemporary hair samples of adult non-consumers were analyzed as negative controls.

Sex could be determined in only eight mummies (one female and seven male adults); two other mummies corresponded to children and one to an infant (Table 1). Dates for the mummies studied have not been determined directly. In the absence of such direct dates, associated cultural periods were determined from features of contextual elements, mainly style of co-occurring pottery (Berenguer et al., 1986; Tarragó, 1989). Assignments could only be made in 21 cases due to the lack of relevant contextual information for the other mummies (Table 1).

The funerary context of mummies included one or more of the following types of objects: arrows, axes, baskets, bones, bowls, bows, boxes, ceramic objects, chisels, chunks of gemstones, flutes, gemstone beads, hammers, hats, hole punches, metal or gemstone ornaments, needles, necklaces, pieces of raw metals (copper, gold), pigments, squashes, snuffing paraphernalia (bags, mortars, pestles, snails, spatules, trays, tubes), spoons, textiles, threaders, vases, vegetable residues, and urns. A context diversity index was considered a general proxy for social and wealth status of the mummy, and was defined as the number of types of objects among those mentioned above found with the mummy (maximum possible value of index = 34). Number of types of objects was used instead of number of objects because the latter figure is in many cases ill-defined, only a plural being mentioned in field notes for some objects. Additionally, although the relative cultural value of objects in SPA tombs has not been assessed quantitatively, the funerary context was examined for the presence of necklaces made of gemstones (typically turquoise) or ignimbrite, another proxy that can be assumed to be related to social and wealth status. A list of contextual objects was available for 41 of the mummies studied (Table 1).

2.2. Chemicals

Methanol and chloroform were high performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany or JTBaker, USA). Nicotine was purchased from Sigma–Aldrich (St Louis, MO, USA). Bufotenine was generously provided by Prof. Maria L. Dos Santos from Instituto de Química – Universidade de Brasília, Brasil.

2.3. Preparation of hair samples

Hair strands (ca. 100 mg) were cut into 1 mm segments, washed with 3 × 3 mL of chloroform (15 min each time) and filtered through a composite made with one layer of 0.20 mm steel mesh and five layers of 0.25 mm steel mesh (Millipore Corporation, Billerica, MA, USA) using a 5 mL glass syringe Ultrafit (Henke, Sass Wolf GMBH, Germany). The washed hair was finely pulverized by placing it inside 1.8 mL stainless steel microvials with polyethylene flange caps and containing 25 stainless steel 1-mm diameter beads, and agitating it for 10 min in a bead beater (Mini-Beadbeater-96; Biospec Inc., Bartlesville, OK, USA). The pulverized hair samples were macerated in 500 μL chloroform with periodical vortexing at ambient temperature for 72 h, and the suspension filtered through cotton wool placed at the tip of a Pasteur pipette using an additional 500 μL aliquot of chloroform. The remaining hair was dried under nitrogen flow and further macerated in 1 mL of methanol with periodical vortexing at ambient temperature for 72 h. The methanolic extracts were filtered through cotton wool placed at the tip of a Pasteur pipette using an additional 1 mL aliquot of methanol. All extracts were first collected in 2 mL amber vials with teflon-lined screw caps. Aliquots were successively transferred to a 300 μL glass insert within an amber vial, from which they were taken to dryness by means of a nitrogen flow; this operation minimized the quantity of residue retained in the vessel walls. For gas chromatography/mass spectrometry (GC/MS) analysis in the scan mode (all ions produced in the mass detector are simultaneously detected), the extracts were reconstituted in 20 μL and for GC/MS analysis in the selective ion monitoring (SIM) mode (only specific ions are detected, thus enhancing the selectivity and sensitivity of analyses), the extracts were reconstituted in 15 μL.

2.4. GC/MS analysis

GC/MS analysis was performed with a Shimadzu model GCMS-QP 2010 Ultra gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a Rtx-5MS Crossbond 5% diphenyl - 95% dimethyl polysiloxane (Restek, Bellefonte, PA, USA) capillary GC column (30 m length, 0.25 mm I.D., 0.25 μm film thickness). The GC was operated in the splitless injection mode; injection volume was 1 μL or 5 μL for scan and SIM modes, respectively. The column temperature was held at 30 °C for 3 min, raised at 25 °C/min to 230 °C, and maintained for 10 min at 230 °C. The carrier gas was helium at a flow rate of 1.3 mL/min. The mass spectrometer was used in the electron impact ionization mode (70 eV) with an emission current

Table 1
Contextual details of hair samples of mummies from San Pedro de Atacama analyzed by GC/MS for the presence of bufotenine and nicotine. Bufotenine was not found in any of the samples; nicotine was found in methanolic extracts of some of the samples, as shown.

Site	Mummy nr.	Sex/age	Cultural period[a,b]	Presence of snuffing paraphernalia[c]	Diversity of funerary context[b,d]	Presence of gemstone necklace	Presence of nicotine[e]
Catarpe 2	1808		Yaye or Solor		2		+
Catarpe 2	1821		Yaye or Solor		3		+
Catarpe 2	1831		na		1		nd
Catarpe 2	1844		na		3		+
Catarpe 2	1890		na		3		+
Catarpe 2	2956	Male adult	na		2		+
Catarpe 2	2968		na		2		+
Catarpe 2	2976	Child	na		8		nd
Catarpe 2	2987		Yaye or Solor		7	Yes	nd
Catarpe 2	2994		na		7		nd
Catarpe 2	2995		na		4	Yes	+
Catarpe 2	2998	Female adult	Yaye or Solor		3		nd
Catarpe 2	3004	Male adult	Yaye or Solor		3		+
Catarpe 2	3010		na	(Yes)	6		nd
Catarpe 2	3011	Male adult	Yaye		11	Yes	+
Catarpe 2	3018	Male adult	Yaye or Solor		20		+
Catarpe 2	3024	Child	na		1		+
Catarpe 2	3026	Male adult	Yaye or Solor		5	Yes	nd
Catarpe 2	3027		na		na		+
Catarpe 2	3032	Male adult	Yaye or Solor		4	Yes	+
Catarpe 2	3033		na		na		nd
Coyo Oriente	3918		na	Yes	8		+
Coyo Oriente	3926		na		5		+
Coyo Oriente	3928		na		3		nd
Coyo Oriente	3935		Quitor or Coyo	Yes	14		+
Coyo Oriente	4091		na		5		+
Coyo Oriente	5333		Sequitor	Yes	14		+
Quitor Conde Duque	1187		na		na		+
Quitor Conde Duque	1331		na		na		+
Quitor 1	891		Yaye or Solor	(Yes)	9	Yes	+
Quitor 1	3435		Yaye or Solor		7	Yes	nd
Quitor 1	3448		Yaye or Solor	Yes	11	Yes	+
Quitor 2	58		Sequitor/Quitor		4		+
Quitor 2	68		Quitor or Coyo	Yes	5		+
Quitor 2	3693		na		na		+
Quitor 2	3785		na		10		nd
Quitor 3	72		na	(Yes)	7		+
Quitor 6	2432		na		na		+
Quitor 6	2511		Quitor or Coyo	Yes	18	Yes	nd
Sequitor Alambrado Oriental	74		Quitor		11	Yes	+
Solcor Nueva Población	4768		na		na		+
Solcor Nueva Población	4769		na		na		+
Solcor Nueva Población	4770		na		na		+
Solcor Nueva Población	4773		na		na		nd
Solcor Nueva Población	4780		na		na		nd
Solcor Nueva Población	4784		na		na		nd
Solcor Nueva Población	4786		na		4		+
Solcor Nueva Población	4788		na		1		nd
Solcor 3	T2-986		Coyo	(Yes)	11	Yes	+
Solcor 3	T86-2930		na		na		nd
Solor 3	10		na		4		+
Solor 3	24	Male adult	Quitor or Coyo	Yes	14		nd
Solor 3	29	Infant	na		2		nd
Solor 3	30		Sequitor/Quitor		3		nd
Toconao Oriente	T15		na		na		nd
Toconao Oriente	T19		na		na		+

[a] Cultural phases: Sequitor (Late Formative Period), Quitor (initial Middle Period), Coyo (late Middle Period), Yaye (initial Late Intermediate Period) and Solor (final Late Intermediate Period). Some archaeological sites contain more than one cemetery belonging to different cultural phases.
[b] na = not available.
[c] In parenthesis: presence of snuffing paraphernalia other than snuff trays.
[d] Number of types of objects found in the funerary context. Maximum is 34.
[e] nd = not detected.

of 250 μA. The temperatures of the injection port, ion source and transfer line were 250 °C, 250 °C and 280 °C, respectively. The instrument was operated in the scan or SIM modes.

Evidence from other regional studies suggested the possible consumption of nicotine or tryptamine derivatives; hence, the analytical methods were oriented toward finding evidence for the presence of these compounds. Since both nicotine and tryptamines are metabolized in the body, the SIM mode program also included characteristic ions for their main metabolites (Fig. 1).

2.5. Validation of GC/MS method

Calibration plots were made with GC/MS peak areas obtained with solutions of pure nicotine or bufotenine against the

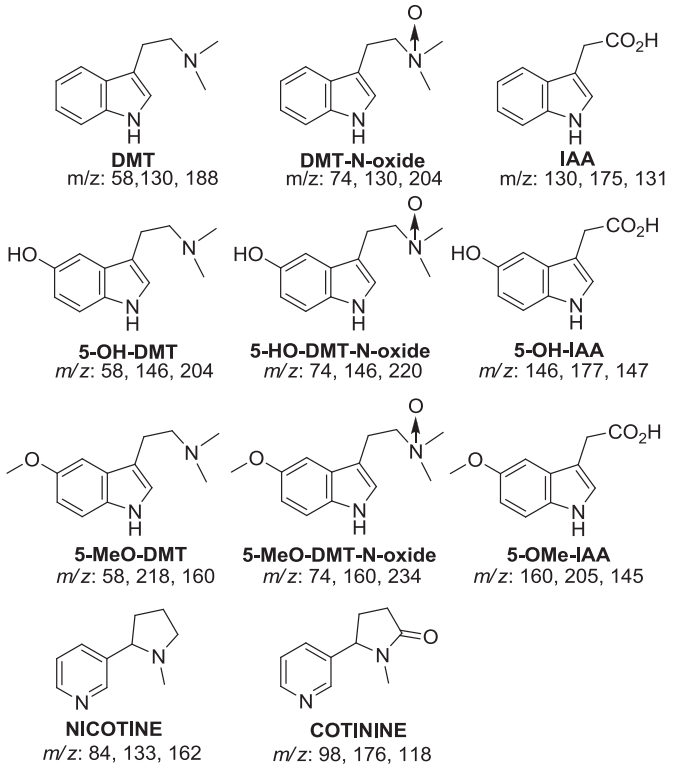

Fig. 1. Main products of transformation of N,N-dimethyltryptamine (DMT), 5-hydroxy-DMT (bufotenine), 5-methoxy-DMT and nicotine in the mammal body, and the main ions produced in the mass spectrometer.

concentrations of the analytes (ranges: 0.5–500 ng for nicotine and 10–500 ng for bufotenine). Above 10 ng for nicotine and 20 ng for bufotenine, calibration plots were linear (correlation coefficient of the regression lines: $r^2 = 0.992$ for nicotine and 0.995 for bufotenine). Experiments in which hair of non-consumers with added nicotine or bufotenine was submitted to the complete extraction and quantitation procedures led to mean recoveries of ca. 90% for nicotine in the range 10–500 ng nicotine/100 mg hair and ca. 75% for bufotenine in the range 20–500 ng bufotenine/100 mg hair.

Identification of nicotine was achieved through the analysis of the gas chromatograms obtained from injections of the methanolic extracts of mummy hair in the SIM mode, which monitored the two most intense spectral peaks of nicotine, at *m/z* 84 and 133. A positive identification of nicotine was defined when the chromatogram of the sample showed a peak falling within the range of pure nicotine (mean = 9.70 min; range = 9.66–9.72 min), and the ratio of ions *m/z* 133 to *m/z* 84 was within two standard deviations of the mean of all nicotine samples analyzed (10 for calibration curves, 10 for recovery experiments, 35 hair samples and 4 instrumental replicates of positive control).

3. Results

The analysis of the adult hair samples of a current consumer of *Anadenanthera colubrina* and tobacco failed to show the presence of dimethyltryptamines or their derivatives but did show the presence of nicotine. On the other hand, analysis of the contemporary hair samples of adult non-consumers did not show the presence of either dimethyltryptamines nor nicotine or their derivatives.

The 56 ancient adult hair samples were tested for the presence of tryptamines and nicotine derivatives. The GC–MS results were negative for both types of alkaloids in chloroform washings and extracts, and also negative for tryptamines in methanol extracts; some methanol extracts showed the presence of nicotine (Table 1). Thus, although chromatograms of methanolic extracts of hair samples were quite complex, a peak at the retention time of nicotine could be discerned for which the ratio of *m/z* 84/133 fell within the values of pure nicotine (Fig. 2). No peaks corresponding to tryptamine derivatives were found, either in the scan or SIM modes.

Nicotine was found in 35 samples, assigned to the Late Formative (1 of 1 sample from this period), Late Formative or Middle (1 of 2 samples from either of these periods), Middle (4 of 6 samples from this period) and Late Intermediate periods (8 of 12 samples from this period), or without assignment to period due to lack of contextual information (21 of 35 samples unassigned to period) (Table 2). The proportion of mummies with and without nicotine in their hair did not differ between tombs with and without snuffing trays (multiple proportions test: chi-squared = 0.4388, d.f. = 1, $P = 0.534$) nor between tombs with and without snuffing paraphernalia (multiple proportions test: chi-squared = 0.872, d.f. = 1, $P = 0.354$). The diversity of the funerary context – measured in terms of the number of types of objects – did not differ between tombs with mummies with or without nicotine in their hair (Mann–Whitney test, $U = 187$, $P = 0.838$). The proportion of mummies with and without nicotine in their hair did not differ between tombs with and without necklaces with gemstone or ignimbrite beads (multiple proportions test: chi-squared = 0.000318, d.f. = 1, $P = 0.986$).

4. Discussion

4.1. Absence of tryptamines

The negative results from the analysis of tryptamines in the hair of the mummies studied lead to the following questions: is the analytical method employed trustworthy – i.e., could tryptamines be present and not be detected? Could tryptamines have been consumed but not have been incorporated into the hair follicle, either due to rapid metabolization or to their molecular structural features? Could tryptamines have been consumed and incorporated into the hair follicle and have degraded since the time intake took place? In other words, the ability to detect tryptamines in the hair of potential consumers depends on the quality of the analytical and sampling methodologies employed, the stability of tryptamines prior to incorporation into the hair follicle, the affinity of tryptamines for the hair follicle, and the stability of the tryptamines within the hair follicle.

The GC/MS method developed shows sensitivity and recovery data which compare well with that reported in other studies of alkaloids in archaeological hair samples where alkaloids have indeed been found (Cartmell et al., 1994; Musshoff et al., 2009). On the other hand, the sampling methodology was not optimal. Segmental analyses of hair samples of drug users show that drug concentration decreases with increasing distance from the root of the hair (Rothe, 1997). In the present study, samples were not necessarily withdrawn from the optimal position within the hair fiber but rather from whatever hair was visible and available in the wrapped mummy.

The degradative metabolism of dimethyltryptamines (DMTs) in mammals occurs primarily via oxidative deamination catalyzed by monoaminooxidase-A and N-demethylation catalyzed by cytochrome P450 to produce DMT-N-oxides and indole-3-acetic acids (IAA), respectively, as major products, the latter probably arising from oxidative deamination of N-methyltryptamines (NMTs) (Yu et al., 2004; Barker et al., 1981). Although the effects of psychoactive tryptamines are dependent on dose and route of administration (Shen et al., 2010), psychedelic experiences tend to be of short

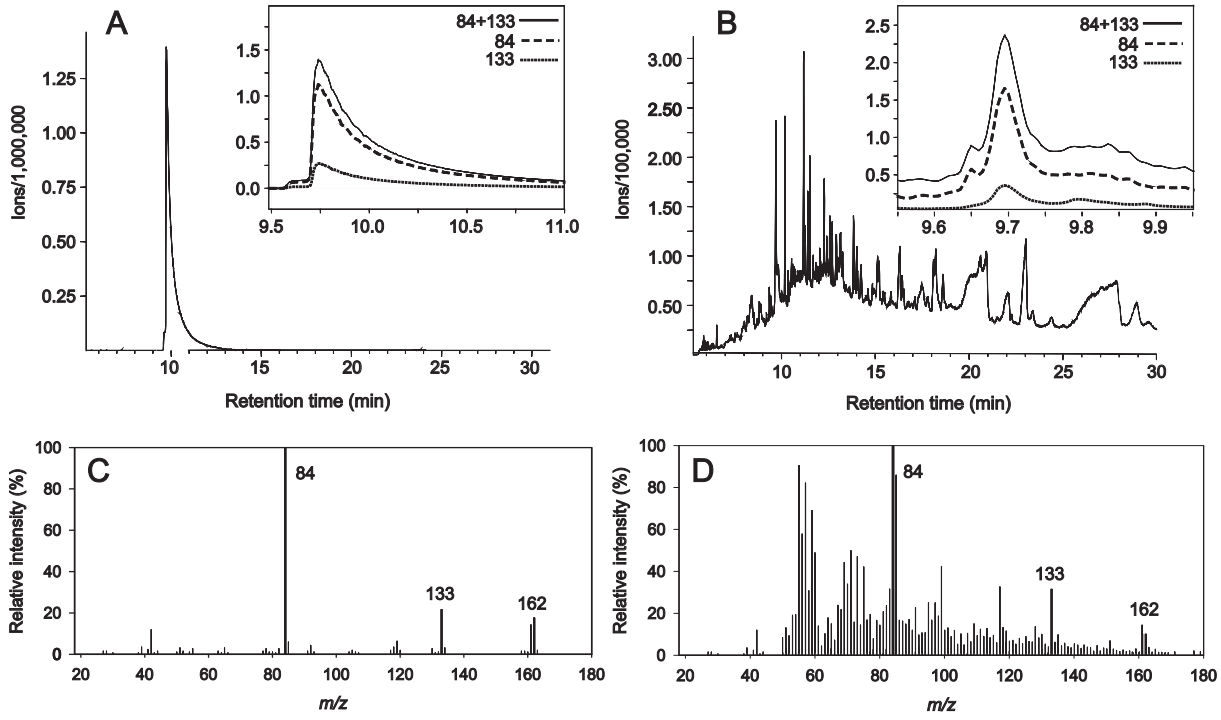

Fig. 2. Analysis of nicotine in the hair of mummies. A: Scan chromatogram of pure nicotine (50 ng) with inset showing SIM chromatogram with two main ions (*m*/*z* 84 and 133); B: Scan chromatogram of methanolic extract of hair sample Quitor Conde Duque 1187 with inset showing the SIM chromatogram with two main ions of nicotine (*m*/*z* 84 and 133). C: Mass spectrum of nicotine; D: Mass spectrum of peak in chromatogram B at the retention time of nicotine.

duration presumably due to fast and effective metabolization (Ott, 2001a,b), a factor that works against their accumulation in the human body and hence the possibility of detecting them.

From a structural viewpoint, lipophilicity and basicity are essential molecular features which favor the incorporation of a molecule into the hair matrix (Pötsch et al., 1997a). While DMT-N-oxides and IAAs are unlikely to get incorporated into the hair follicle on account of their polar nature, NMTs are probable candidates for accumulation in hair. However, these latter compounds are intermediates which are presumably accumulated in such low amounts that their concentration might well be below the detection limit of the current method. On the other hand, hair melanin content has been shown to affect the incorporation into hair of alkaline molecules such as alkaloids. Thus, basic molecules show significant in vitro affinity for melanin (Pötsch et al., 1997b; Claffey et al., 2001) and concentration of alkaline drugs in pigmented hair is higher than in non-pigmented hair (Uematsu et al., 1990; Sato et al., 1993; Mizuno et al., 1993; Rothe et al., 1997). The hair of natives from SPA (mummies as well as their present-day descendants) are black (with the exception of some older individuals whose hair is greyish), suggesting a capacity to accumulate alkaloids.

If DMTs are in fact incorporated in hair, their chemical transformation in the hair follicle is also possible. Hair fibers may suffer microbiological degradation (Wilson et al., 2007), and compounds contained in hair may undergo diagenetic transformations; for example, long-term effects of weather factors such as sun, rain and wind have been shown to damage hair with subsequent impacts on drug concentration in it (Skopp et al., 1997). Given the prevailing extreme desertic conditions in SPA, hair of mummies from SPA has not been exposed to such microbiological and climatic factors. Hence, diagenetic transformations on account of such factors seem unlikely.

Independently of the possible causes for the absence of DMTs from the ancient hair samples analyzed, it should be noted that the contemporary hair sample of a consumer of *A. colubrina* and tobacco contained nicotine but not DMTs or their derivatives. Thus, the absence of DMTs in the hair of mummies should not be taken as robust evidence for lack of their consumption.

Table 2
Summary of presence of nicotine in hair samples studied.

Cultural phase/Period		Total samples	Samples with nicotine
Sequitor	Late Formative	1	1
Sequitor or Quitor	Late Formative or Middle	2	1
Quitor	Middle	1	1
Quitor or Coyo	Middle	4	2
Coyo	Middle	1	1
Yaye	Late Intermediate	1	1
Yaye or Solor	Late Intermediate	11	7
Unknown	Unknown	35	21
Total		56	35

4.2. Presence of nicotine

The presence of nicotine in archaeological samples may be attributed to sample contamination from, for example, people conducting archaeological excavations, various personnel manipulating stored objects and also visitors to museums who may be tobacco smokers. In hair analyses, contamination may be presumed of minor importance if analysis of the external washings of the hair reveals the absence of nicotine and also of cotinine, its main decomposition and metabolic product (Gorrod and Schepers, 1999; Tyrpien et al., 2003). In the present case, chloroform washings of the hair whose methanolic extracts showed presence of nicotine did not contain nicotine nor cotinine, thus showing that consumption of nicotine occurred during the life time of the individual.

Nicotine has been found in the hair of pre-Columbian mummies from as far back as 1095 A.D. (Musshoff et al., 2009) and also in smoking pipe residues from sites in North America dated as early as 300 B.C. (Rafferty, 2002, 2006; Rafferty et al., 2012; Tushingham et al., 2013); in other words, nicotine is stable enough to withstand the passage of at least one millennium within a hair follicle and two millennia in a pipe residue.

The main sources of nicotine in the Americas are species of the genus *Nicotiana*, i.e. tobacco. The use of tobacco by native populations for over four millennia has been documented (Winter, 2000). Different cultures have consumed tobacco in a variety of ways, such as by smoking, chewing, eating, snuffing or through enemas (Wilbert, 1987). The mode of consumption is usually related to the type of effect pursued, i.e. whether used in group ceremonies, for individual healing purposes, or for the achievement of particular states of mind (Wilbert, 1987), and it is likely based on the dose-dependent effects of nicotine, e.g., in small quantities it is a stimulant and painkiller while in large doses it produces visions, trance and catatonia (Wilbert, 1994), and also its rate of incorporation into the bloodstream and accessing the brain (Illum, 2004).

The analysis of hair from mummies from SPA showed that nicotine was present, overall, in 63% of the samples studied (Table 2). The mummies studied encompassed over one millennium of cultural development. Furthermore, the data obtained shows that nicotine consumption occurred with similar frequencies during the periods studied. Thus, after an eventual exact dating of their contexts, mummies presently ascribed to the Late Formative (ca. 100 B.C.–400 A.D.) and Middle (400–950 A.D.) periods will be assigned to one of the two periods, in which case prevalence of nicotine in samples from the Late Formative period could vary between 50 and 100% and prevalence in those from the Middle period could vary between 57.1 and 71.4%, whereas prevalence of nicotine consumption amounted to 67% in the Late Intermediate period (950–1450 A.D.).

The means of consumption of nicotine has experienced a diachronous variation in SPA. During the Late Formative period, pipes are found in SPA cemeteries, the oldest of which show the style of the San Francisco complex from NWA (ca. 650 B.C.–300 A.D.). The increased influence of the Tiwanaku state during the Middle period brought important changes within the SPA society. The archaeological record in SPA cemeteries shows that patterns of exchange of goods were altered as well as the partners of those exchanges (Uribe, 2002; Stovel, 2005); most importantly, religious and ceremonial practices and their associated paraphernalia experienced a profound transformation (Torres, 1999). Thus, a change is observed in the mode of administration of psychoactive substances, from predominantly smoking in pipes to snuffing in trays, the latter mode following altiplanic traditions (Llagostera, 1996; Torres, 1999; Berenguer and Dauelsberg, 1989; Thomas et al., 1984). Interestingly, near the Pacific coast to the west of SPA, a tomb containing ceramic objects ascribed to the early Middle period (Tarragó, 1989; Berenguer et al., 1986) contained two snuff trays and two stone pipes (Gili Hanish et al., 2009), suggesting the concurrent use of both consumption modes.

The present results unambiguously show consumption of nicotine during this period, most likely from tobacco (see below). It is not surprising that peoples in SPA moved from tobacco smoking to snuffing since drugs administered nasally may reach a target in the brain faster and to a higher extent than through other routes of administration (Illum, 2004). Thus, drugs absorbed by the rich network of blood vessels in the nasal cavity may pass directly into the systemic circulation and further transfer across the blood–brain barrier, the mechanism being particularly efficient in the case of non-polar drugs (Illum, 2002). It should be noted that although tobacco is usually associated to smoking pipes, the use of tobacco snuffs was documented soon after the time of contact and ethnographic evidence abounds on its prevalence until modern times (Wilbert, 1987).

The precise source of nicotine in mummies from SPA cannot be ascertained since both the area around SPA and the surrounding areas in NWA and Bolivia are hosts to several species of *Nicotiana*; in fact, west central South America is considered the place of origin of the subgenera *rustica* and *tabacum* of the genus *Nicotiana* (Goodspeed, 1954). Additionally, nicotine may have been obtained from non-*Nicotiana* species growing in nearby regions with described interactions with SPA, e.g., *Dunalia spinosa* (Solanaceae) (Espinoza et al., 2012).

It may be argued that the psychotropic molecules toward which the present analyses were directed were not the only ones to have been consumed by prehispanic societies in SPA. Thus, it seems unlikely that activities as important as the consumption of psychoactive substances depended for several hundred years on just a few botanical and geographical sources. One alternative is that prehispanic societies in SPA obtained psychotropic substances not from present-day Bolivia or Argentina, the closest regions where DMT sources grow abundantly, but from the North of Chile. If such were the case, candidate sources for psychotropic substances are the cacti *Echinopsis pachanoi*, of extended use by prehispanic societies of Peru (Glass-Coffin, 2010) and which could have been obtained through exchanges via the Pacific coast, and *Browningia candelaris*, which has been shown to accumulate psychoactive phenylethylamines (Echeverría and Niemeyer, 2012) and grows in the prepuna area of Northern Chile. It also seems likely that prehispanic peoples in SPA screened the local flora for the presence of hallucinogenic substances which could substitute the imported substances. If this were the case, hitherto unknown psychotropic substances from the local flora may have been used and their eventual presence in hair be undetected by the methods employed in the present study; the phytochemical exploration of the flora of Northern Chile in search for psychotropic substances would be desirable for future evaluations of ancient hair samples or residues in ceremonial archaeological objects from SPA.

The statistical analyses of the data show that the frequency of occurrence of nicotine in the hair of mummies was not related to the presence or absence of snuffing paraphernalia in the burial suggesting that, if shamanic activities of individuals were associated to the presence of snuffing paraphernalia amongst their grave goods, then consumption of psychoactive alkaloids was not performed only by shamans. Furthermore, the frequency of occurrence of nicotine in the mummy's hair was not related to either of the two proxies designed to reflect social and wealth status. If burials in SPA cemeteries represent a cross section of the population (i.e., if cemeteries are not socially segregated), then it is likely that consumption of psychoactive compounds was practiced by members of society indistinctive of their social and wealth status.

During the period of Tiwanaku influence in SPA and neighboring areas, indirect data suggests consumption of dimethyltryptamine-derived compounds which were derived from seeds of *A. colubrina* (Torres et al., 1991; Gili Hanish et al., 2009), a tree species growing in south and southwestern Bolivia and northwestern Argentina but not occurring in SPA. Since psychoactive preparations often involve a mixture of substances which interact to create in the consumer a range of effects during an extended period of time (Wilbert, 1987; Torres and Repke, 2006), it is not unlikely that some of the consumers of nicotine were also consumers of other alkaloids such as DMTs, at least during the times when both types of compounds were widely accessible at SPA. If consumption of tryptamines had indeed occurred, then it is likely that the prevalence of alkaloid consumption could exceed that found in the present work.

In summary, the present results show the continuous consumption of nicotine in SPA, from the Late Formative period to the Late Intermediate period, in spite of profound changes in the administration technology, which changed from smoking pipes to snuffing trays. The results also support the idea that consumption of psychoactive alkaloids in SPA was performed by members of the society at large, irrespective of their social and wealth status.

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