Waking-promoting action of Yerba Mate

(Ilex paraguariensis)

Ação promotora de vigília da erva mate (Ilex paraguariensis)

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ABSTRACT

Objectives: Yerba mate (Ilex paraguariensis, Ip) consumed as an infusion (tea), is a traditional and widespread beverage from South America. Even though a waking-promoting effect of Ip is suggested in folklore and traditions, there are no studies that have analyzed the effect of Ip on wakefulness and sleep. Hence, the aim of the present report is to study, for the first time, the effects of Ip on wakefulness and sleep. Methods: Adult cats were prepared for polysomnographic recordings in semi-restricted conditions for 4 hours/day. The animals were recorded after oral Ip (10 and 30%) administrations in control and sleep deprived conditions. We analyzed different parameters of wakefulness and sleep, and performed a quantitative analysis of the electroencephalogram (EEG). Results: Ip infusion produced a dose-dependent increase in wakefulness and a decrease in non-REM sleep (NREM). Comparing to control infusion, during a sleep deprivation protocol a smaller number of stimuli were required to keep the animal awake under the effect of Ip. Furthermore, the sleep rebound that followed the sleep deprivation protocol, was significantly reduced. The waking-promoting effect of Ip infusion administered daily, was maintained for seven consecutive days, and the abrupt cessation of Ip administration did not result in an increase of sleep. EEG quantitative analysis suggests that Ip produces more alert or attentive wakefulness and shallow sleep episodes. Conclusions: We demonstrated that Ip is a natural product with activating effects. This preclinical study may be the basis for new natural treatments to reduce sleepiness.

Keywords: caffeine, medicinal plants, natural products, sleep stages, wakefulness.

RESUMO

Objetivos: Erva mate (Ilex paraguariensis, Ip), consumida como uma infusão, é uma bebida tradicional e difundida na América do Sul. Embora os efeitos promotores da vigília da Ip sejam sugeridos tradicionalmente e folcloricamente conhecidos, não existem estudos que tenham analisado seus efeitos sobre a vigília e o sono. Assim, o objetivo do presente trabalho foi estudar, pela primeira vez, os efeitos da Ip sobre a vigília e o sono. Métodos: Gatos adultos foram preparados para registros polisonográficos em condições de semi-restricção por 4 horas por dia. Os animais foram registrados após administração oral de Ip (10 e 30%), tanto em condições de controle e de privação de sono. Analisamos diferentes parâmetros de vigília e sono e realizamos análises quantitativas do eletroencefalogramp (EEG). Resultados: Administração de Ip induziu um resposta dose-dependente na vigília e uma decaimento no sono não-REM (NREM). Comparando às infusões controle, durante o protocolo de privação de sono, um pequeno número de estímulos foram necessários para manter o animal acordado sob o efeito de Ip. Além disso, o reboque de sono que segue a privação de sono foi significativamente reduzido. Os efeitos promotores da vigília obtidos pela administração de Ip foram mantidos por sete dias consecutivos e o término abrupto da administração de Ip não resultou em aumento do sono. Análises quantitativas do EEG sugerem que Ip induz mais alerta e vigília atenta e episódios de sono superficiais. Conclusões: Nós demosnstramos que Ip é um produto natural com efeitos estimulantes. Este estudo pré-clínico pode servir como base para novos tratamentos naturais para reduzir a sonolência.

Descritores: cafeína, fases do sono, plantas medicinais, produtos naturais, vigilia.

INTRODUCTION

Compared with previous decades, we assist a time characterized by a reduction in sleep time with frequent sleep disorders in urban populations1,2. A lack of adequate sleep time with the consequent accumulation of a “sleep debt”, the excessive sleepiness disorders, as well as increasingly more demanding daily activities, have popularized the use of waking-promoting medication (stimulants)2,3.

Caffeine consumed as coffee, is the most common stimulant3. However, in south-American countries such as Uruguay, Argentina, Paraguay and Brazil, the use of coffee as a stimulant has been partially replaced by the use of yerba mate infusion4,5.

Yerba mate leaf is the raw material of three different types of infusions: the “mate or chimarrão”, “mate tea” and “tereré” which vary in water temperature, the way it is prepared and con-
sumed. This leaf is from the *Ilex paraguariensis* (Ip) tree, that belongs to the family Aquifoliaceae (43). The processing of the leaves of *Ip* consists on the grinding, drying, fragmentation separation of the leaf stalks. Flavonoids, alkaloids, tannins, saponins, vitamins and minerals such as iron, manganese, potassium are among the components of *Ip*. The main alkaloids are caffeine and theobromine. Caffeine is present in concentrations of 1-2% of the dry weight, while there is 0.3 to 0.9% of theobromine (46). Along with the ongoing traditional usage of yerba mate beverages, *Ip* has made its way to beers, creams, candy, and other non-traditional uses. In the last decade, it has reached supermarkets in the form of energy drinks in United States and is been sold in Europe in combination with other herbs as an energy tea or as a weight reduction aid (9).

*Ip* has positive effects on human health and is considered a medicinal plant. It has antioxidant, anti-inflammatory properties, hypcholesterolemic, antiparasitic, antimutagenic and anticarcinogenic potential, as well as weight reducing effects (43-78). Among the few studies regarding the effects of the *Ip* on the central nervous system (CNS) and behavior, it has been reported that *Ip* improves motor deficits in an animal model of Parkinson’s disease, as well as memory and learning in rats evaluated with behavioral tests (9,10). In spite of the traditional belief that yerba mate is a stimulant beverage, there are no studies concerning the effects of *Ip* on wakefulness and sleep. Therefore, the aim of the present study was to analyze in an animal model the effects of *Ip* on the natural sleep-wake cycle, the drowsiness caused by sleep deprivation, and the post-deprivation sleep rebound. We also studied the effect of the daily administration of *Ip* on sleep (tolerance), and the effect of an acute cessation in the administration (abstinence). Finally, we performed a quantitative analysis of the electroencephalogram (EEG) after *Ip* administration.

**METHODS**

**Animals**

Three adult cats (*Felis Domesticus*) were used in this study. The animals were housed with food and water available *ad libitum*, and maintained under normal laboratory conditions (temperature 21-23°C, 12h day-night cycle, lights on at 7:00 AM). All experimental procedures were conducted in accordance with the “Guidelines for the care and use of laboratory animals” (8th edition, National Academy Press, Washington D. C., 2011). The Animal Care Committee of the Universidad de la República approved the experimental protocol (071140-000777-10). Adequate measures were taken to minimize pain, discomfort or stress of the animals. In addition, all efforts were made in order to use the minimal number of animals necessary to produce reliable scientific data.

**Surgical procedures**

The following surgical procedures have been widely used by our group (11-13). The animals were chronically implanted with electrodes to monitor the states of sleep and wakefulness. Prior to being anesthetized, each cat was premedicated with xylazine (2.2 mg/kg, i.m.), atropine (0.04 mg/kg, i.m.) and antibiotics (Tribrissen®, 30 mg/kg, i.m.). Anesthesia, which was initially induced with ketamine (15 mg/kg, i.m.), was maintained with a gas mixture of isoflurane in oxygen (1-3%). The head was positioned in a stereotaxic frame and the skull was exposed. In order to record the EEG, stainless steel screw electrodes (1 mm diameter) were placed on the surface (above the dura matter) of frontal, parietal, occipital cortical regions. Bipolar electrodes were implanted in both lateral geniculate nuclei (LGN; A 6.0, L 10.5, H 3.5 according to Berman and Jones atlas (14)) in order to monitor the ponto-geniculo-occipital (PGO) waves.

The electrodes were connected to a Winchester plug, that together with two plastic tubes (which were used to maintain the animal’s head fixed without pain or pressure) were bonded to the skull with acrylic cement.

At the end of these surgical procedures an analgesic (Buprenex®, 0.01 mg/kg, i.m.) was administered for 48 hours. Incision margins were kept clean and a topical antibiotic was administered on a daily basis.

**Habituation**

The animals were gradually adapted to the semi-restricted experimental conditions (see below) for a minimum of one month, until they habituate to stay during 4 hours per day in the recording set-up. Due to the long adaptation period, these animals felt asleep as soon as they were located in the recording set up; no signs of stress were observed in these animals.

**Preparation of yerba mate solution**

*Ip* solution was prepared as in previous studies (15,16), which reproduce archetypal yerba mate infusions. *Ip* samples were taken from the commercial lot 2-170708 from Canarias S.A. We use *Ip* solutions at different concentrations: 0, 2.5, 5, 10 and 30% obtained by placing the adequate weight of *Ip* in distilled water; the solution remained at 70°C for 15 minutes. Thereafter the solution was filtered and was allowed to cool at room temperature (20-22°C) before administration.

**Ip** administration

A total volume of 20 ml of *Ip* solution or vehicle was administrated orally by means of a syringe applied directly to the mouth (without the need of gavages) over a period of 5 minutes.

**Quantification of caffeine concentration in the *Ip* samples**

The content of caffeine in the samples of *Ip* was determined using a technical analysis of high precision liquid chromatography with detection by arrangement of diode array detection (HPLC-DAD, 1525 pump, automatic injector 717 plus and DAD 2998, Waters, MA, USA), a chromatographic column C-18 (particles of 5 μm, 150 mm x 34.6 mm; Phenomenex, USA). The mobile phase was buffer acetate (pH 3.65) and methanol (75:25). Caffeine content was determined at 272 nm using a standard solution of caffeine (C0750, Sigma-Aldrich). In the preparation of *Ip* 10%, the concentration of caffeine detected was 0.065 mg/ml. This concentration corresponded to 1.3 mg of caffeine in the
volume (20 ml) administered. In Ip 30% the concentration of caffeine was 0.20 mg/ml, which corresponded to 4 mg for the administered dose.

Experimental sessions
Experimental sessions of 4h in duration were conducted between 11 A.M. and 3 P.M. in a temperature-controlled environment (21-23°C). All animals had free access to water and food until 1h prior to the beginning of each recording session. During these sessions (as well as during adaptation sessions), the animal’s head was held in a stereotaxic position by four steel bars that were placed into the plastic tubes while the body rested in a sleeping bag (semi-restricted condition).

During experimental sessions the EEG (through cortical bipolar recordings), LGN electrogram and electromyogram (EMG, by means of acutely placed bipolar electrodes on the skin) of the nuchal muscle were recorded.

Bioelectric signals were amplified (×1000), filtered (0.1 or 10 Hz high-pass, 100 Hz low-pass), sampled (512 Hz, 2^16 bits) and stored in a PC using the Spike 2® software (Cambridge Electronic Design).

Experimental design
Three experimental series were conducted; after each experiment, the animals were allowed to rest for at least two weeks.

Experiment 1. The recording sessions began following vehicle or Ip (10%) administration. A total of 25 control (distilled water) and 10 Ip (10%) infusions were carried out in three animals. Each day of Ip administration infusions was preceded or followed by 1 or 2 days of vehicle administration. The number of Ip 10% infusions for each animal was 4, 3 and 3. The numbers of vehicle (distilled water) sessions for each animal were 9, 7 and 7.

In one animal a dose-response curve (0, 2.5, 5 and 10%) was performed (3 Ip administration per dose).

Experiment 2. The animals (n = 2) were deprived of sleep for 4 hours in two stages: 1) the animal freely moved (exploring, playing) for two hours, 2) sleep deprivation continued with the animal in semi-restricted condition while polysomnographic recordings were carried out. When the animal showed signs of sleep, mild somesthetic stimuli (by gently fondling the animal as in previous studies[13]) were applied to keep it awake. After the sleep deprivation period the animals were recorded for two hours (sleep rebound condition).

Before sleep deprivation in semi-restricted conditions, Ip solutions (10 or 30% in different animals) or vehicle were administered. Five control and three Ip experiments were performed in each animal.

Experiment 3. In one animal, after a period of 4 days of basal recordings after vehicle administration, Ip (10%) was administered daily for the following 7 days. Basal recording with vehicle administration was also performed daily for other 4 days following Ip administration.

Analysis and data processing
Polysomnographic recordings were analyzed in 10 seconds epochs and behavioral states were determined according to standard criteria[17]. The total time spent in wakefulness, NREM and REM sleep as well as the duration, frequency, number of episodes and sleep latencies were analyzed.

Quantitative analysis of the EEG. We analyzed the power spectrum of the EEG by means of Fast Fourier Transformation (FFT) of the first hour after Ip 10% or vehicle administration, in three recordings of three different animals (nine recordings per treatment). The absolute values of power were obtained from consecutive 10 seconds periods for delta (0.5 to 4 Hz), theta (4.5 to 8.5 Hz), sigma (9 to 14 Hz) and gamma (30.5 to 50 Hz) frequency bands. The delta, theta, and sigma bands during NREM sleep, and the gamma band during wakefulness were compared between Ip and vehicle treatments.

Values were presented as mean ± standard error. The level of significance between groups was analyzed using the Student t test or the one-way analysis of variance (ANOVA) followed by Tukey post hoc test. The null hypothesis was rejected with a p < 0.05.

RESULTS
Effects of Ip administration on sleep and wakefulness
We studied the effects on wakefulness and sleep four hours following Ip (10%) infusion. Figure 1 shows two representative hypnograms of the same animal after administration of vehicle and Ip. Administration of Ip caused a significant increase in waking time and a correlated decrease in total sleep time (see Table 1 and Figure 2). This decrease in sleep time was due to a significant decrease in NREM sleep. The effect of Ip was observed mainly in the first and second hour (Figure 2). There were no significant differences in the other parameters studied (Table 1). As evidenced in Figure 3, the effects of Ip administration were dose-dependent; an increase of the concentration of Ip determined a tendency to increase wakefulness and to decrease NREM sleep.

Figure 1. Representative hypnograms following Ip or vehicle administration. Arrows indicate the moment of Ip 10% or vehicle administration. The hypnograms are from experiments performed in the same animal. NREM, non-REM sleep; REM, REM sleep; W, wakefulness.

Effects of Ip administration on the sleepiness caused by sleep deprivation
Figure 4A shows a representative polysomnographic recording during sleep deprivation. Each time the animal showed
Table 1. Effects of Ip on wakefulness and sleep.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ip (10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wakefulness</td>
<td>117.8 ± 13.3</td>
<td>181.8 ± 17.4*</td>
</tr>
<tr>
<td>NREM sleep</td>
<td>101.3 ± 6.0</td>
<td>49.2 ± 1.3*</td>
</tr>
<tr>
<td>REM sleep</td>
<td>20.8 ± 7.1</td>
<td>9.0 ± 4.6</td>
</tr>
<tr>
<td>Total sleep time</td>
<td>122.1 ± 13.3</td>
<td>58.3 ± 17.3*</td>
</tr>
</tbody>
</table>

Table 2. Effects of Ip on number of episodes and duration of episodes.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ip (10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of episodes</td>
<td>53.3 ± 2.8</td>
<td>38.2 ± 9.3</td>
</tr>
<tr>
<td>Duration of the episode</td>
<td>2.4 ± 0.4</td>
<td>30.7 ± 27.0</td>
</tr>
</tbody>
</table>

Figure 3. Dose-dependent effect of Ip administration. Dose-response curve performed in one animal (3 Ip administrations per dose). Asterisks indicate significant differences between groups (*, p < 0.05, ANOVA and Tukey post-hoc test).

Effects of daily Ip administrations
The waking-promoting effect of Ip was maintained after daily administration during seven consecutive days (Figure 5). The day after Ip administration series, wakefulness returned to basal levels without a sleep rebound.

Effect of Ip on the EEG spectrum
Table 3 shows a quantitative analysis of the power of the frequency components of the EEG records obtained under the effects of Ip or vehicle administration. Compared to their respective controls, we observed a significant increase in gamma power during wakefulness after Ip administration in all the animals analyzed. There was also a significant decrease in delta power in two animals and an increase in theta power in all animals during NREM sleep. The effect on sigma (sleep spindles frequency) power during NREM sleep differed among animals.

DISCUSSION
The present report shows that Ip intake produced a dose-dependent increase in wakefulness and a decrease of NREM sleep time. After 7 days of daily administration, the Ip activating effect was still observed, returning to basal levels of wakefulness after abrupt cessation of the intake. In addition, Ip decreased the number of stimulus needed to keep the animals awake during sleep deprivation, and reduced the sleep rebound that followed the deprivation protocol. Besides, in comparison to vehicle administration, Ip decreased delta and increased theta power of the EEG during NREM sleep, demonstrating that sleep was more superficial (17). Moreover, Ip administration increased gamma power (an index of attentive or alert wakefulness (18)).
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Figure 4. Ip reduces sleepiness caused by sleep deprivation. A: Representative raw polysomnographic recording of an animal during sleep deprivation. It illustrates the EEG, NGL, electrogram and EMG as well as the arousal stimulus. When early signs of sleep appear, a mild somesthetic stimulation was applied to awake the animal (activation of the EEG, and disappearance of PGO waves in the NGL). EEG, electroencephalogram; EMG, electromyogram; LGN, lateral geniculate nucleus; B: Representative hypnograms of an animal during and following the sleep deprivation protocol treated with vehicle and Ip (30%). The stimuli needed to keep the animal awake are shown below. It is readily observed a marked decrease in the number of stimuli after Ip administration. In addition, Ip decreased the amount of the sleep rebound that followed the deprivation protocol. The arrows indicate the moment of the administration of Ip or vehicle. NREM, non-REM sleep; REM, REM sleep; W, wakefulness; C: The chart on the left shows the average number of arousal stimuli recorded after the administration of vehicle (113.6 ± 9.5) and after Ip 10% (60 ± 8.7). On the right, the average number of stimuli after the administration of vehicle (99.2 ± 24.4) and Ip 30% (22 ± 1.1) are shown. Different animals were used for each dose of Ip. Five Ip and three vehicle administrations were performed in each animal. Significant differences between Ip and controls are shown by asterisks (p < 0.05, unpaired two-tailed Student’s t test).

Table 2. Effects of Ip on the sleep rebound that followed a sleep deprivation protocol.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ip (10%)</th>
<th>Control</th>
<th>Ip (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wakefulness</td>
<td>25.7 ± 3.8</td>
<td>46.3 ± 8.3*</td>
<td>30.8 ± 8.1</td>
<td>62.8 ± 13.7*</td>
</tr>
<tr>
<td>NREM sleep</td>
<td>74.6 ± 3.8</td>
<td>59.1 ± 4.3*</td>
<td>69.4 ± 6.5</td>
<td>40.5 ± 11.2*</td>
</tr>
<tr>
<td>REM sleep</td>
<td>19.6 ± 1.0</td>
<td>14.5 ± 5.1</td>
<td>19.7 ± 3.0</td>
<td>16.6 ± 3.7</td>
</tr>
<tr>
<td>Total sleep</td>
<td>94.2 ± 3.8</td>
<td>73.6 ± 8.3*</td>
<td>89.2 ± 8.1</td>
<td>57.5 ± 13.7*</td>
</tr>
</tbody>
</table>

Data was obtained from one animal treated with Ip (10%) and for another with Ip (30%) and its respective controls (vehicle). Five Ip and three vehicle administrations were performed in each animal. The period of analysis corresponded to the two hours that followed the sleep deprivation protocol. Time is expressed in minutes. Asterisks represent significant differences (p < 0.05, two-tailed unpaired Student’s t test) between Ip and vehicle.

during wakefulness. On the contrary, the results on sigma power (that corresponds to spindles frequency) during NREM sleep were inconsistent (differs in the cats that were studied).

Figure 5. Effects of daily administration of Ip. A: Effect on the percentage of wakefulness during sleep deprivation (day 5 to 11) and vehicle (day 1 to 4, and day 12 to 15). Dashed line shows the mean percentage of wakefulness during basal (vehicle administration) recordings; B: Graphic chart representing the percentage of wakefulness (left) and NREM sleep (right) for pre-treatment controls (vehicle), for Ip administration, and during the post-Ip administration days (vehicle). Significant differences between groups are indicated by asterisks. p < 0.05, ANOVA and Tukey tests.

Table 3. Effect of Ip on the EEG during wakefulness and sleep.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ip</th>
<th>Control</th>
<th>Ip</th>
<th>Effect</th>
<th>p</th>
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<tbody>
<tr>
<td>Wakefulness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamma (µV²)</td>
<td>C 1</td>
<td>90.2 ± 121.4*</td>
<td>40.5 ± 8.5</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2</td>
<td>10.1 ± 1.8*</td>
<td>9.7 ± 1.6</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 3</td>
<td>37.0 ± 37.5*</td>
<td>24.6 ± 18.5</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>NREM sleep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta (µV²)</td>
<td>C 1</td>
<td>885.2 ± 71.4*</td>
<td>1193.6 ± 557.4</td>
<td>d</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2</td>
<td>198.9 ± 20.7*</td>
<td>285.1 ± 78.7</td>
<td>d</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 3</td>
<td>1500.3 ± 12.7</td>
<td>1500.7 ± 20.8</td>
<td>-</td>
<td>= 0.87</td>
<td></td>
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<tr>
<td>Theta (µV²)</td>
<td>C 1</td>
<td>1379.3 ± 974.3*</td>
<td>880.3 ± 305.1</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2</td>
<td>234.0 ± 72.6*</td>
<td>99.5 ± 33.2</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 3</td>
<td>554.0 ± 41.5*</td>
<td>244.3 ± 191.5</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>Sigma (µV²)</td>
<td>C 1</td>
<td>771.0 ± 443.9*</td>
<td>600 ± 255.0</td>
<td>i</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 2</td>
<td>67.1 ± 70.4*</td>
<td>101.6 ± 60.8</td>
<td>d</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C 3</td>
<td>300.9 ± 255.8</td>
<td>300.7 ± 37.2</td>
<td>-</td>
<td>= 0.25</td>
<td></td>
</tr>
</tbody>
</table>

Data were taken from the EEG recordings after nine controls (vehicle) and nine Ip (10%) administrations on three cats (3 controls and Ip in each cat). The EEG were analyzed from cortical electrodes located in the following cortices: right parietal-occipital vs. left frontal-parietal (C2); right frontal-parietal vs. right occipital (C2); left parietal vs. left frontal-parietal (C3). The statistical analyses were performed for each animal against its own control by means to the two-tailed unpaired Student’s t test. C, cat; i, significant increase; d, significant decrease.
Limitations of the present study
The chemical composition of the Ip is affected by variations in weather conditions, harvest season and industrial processing. In this study we used a batch of trademark yerba mate; however, we cannot rule out that the use of other types of Ip from another source could produce some differences in the results.

The Ip infusion was administered in a single dose, which differs from the more common form of consumption, the “mate”, in which the product is consumed for several hours with a gradual decrease in the concentration of Ip extract.

We employed the cat as the animal model, because, in addition to our experience with this model, the cat has been considered for a long time the standard animal model for sleep research; this species spends almost fourteen hours per day in sleep, and their sleep episodes are consolidated. However, we do not know whether there are differences between cats and humans in the absorption, metabolism or excretion of the different components of Ip. In fact, there are differences in caffeine metabolism between rodents and humans.

In order to use the minimal number of animals necessary to produce reliable scientific data we made comparisons using the animals as their own control; this approach strengthens the statistical analysis.

Ip components responsible for the activating effect
We demonstrated a clear waking-promoting effect of Ip in normal, sleep-deprived, and somnolent (during sleep rebound) animals, and this effect did not decline after 7 days of daily administration; however, it would be important to study the effects of Ip on tolerance and abstinence for longer periods of time.

Among the compounds of the Ip, caffeine is found in high concentrations, while theobromine as well as theophylline are present in small amounts. The dose of Ip 10% contained 1.3 mg of caffeine, while Ip 30% contained 4 mg. Note that a cup of instant coffee contains 40 to 108 mg of caffeine and a caffeine tablet 40 to 65 mg. If we extrapolate the dose of caffeine used for 60 kg, we would be using approximately 6.5 mg (Ip 2.5%) to 72 mg (Ip 30%). Therefore, the amounts administered are in range used for human consumption.

Caffeine exerts its biological effect by blocking adenosine receptors, an endogenous neuromodulator that plays an important role as a sleep promoter. The cellular effects of adenosine are mediated by four receptor subtypes: A1, A2A, A2B, and A3. In the CNS, the predominant subtypes are A1 and A2, which are most likely associated with the mediation of the physiological effects of adenosine in sleep. It has been observed that during sleep deprivation, extracellular adenosine is accumulated in areas such as the basal forebrain (part of the activating system), causing an increase tendency to sleep. The pharmacological blockade of adenosine receptors with caffeine interferes with the increased tendency to sleep of adenosine accumulation.

Although the known main active stimulant compound of Ip is caffeine, it cannot be excluded that other components may contribute to maintenance of wakefulness. It is also possible that other components may modify the absorption or hepatic metabolism of caffeine, which would alter its effects. In this regard, it is known that marijuana components administered separately have opposite effects on sleep-wake cycle, so the natural marijuana effects depends on the balance between its components. Therefore, it would be important to compare the effects of Ip with equimolar doses of caffeine in order to determine if other components, besides caffeine, promote the arousal effect.

Translational significance of the results
The study of the stimulant properties of Ip and the components that cause such effects, could lend support to the selection and production of new varieties of Ip, and the development of other types of products catering to different consumer needs. For example, varieties without or with low levels of caffeine could be useful in yerba mate consumers with insomnia. Moreover, varieties that enhance the waking-promoting effects of Ip could be an option to reduce sleepiness for people that need to be awake for longer periods of time, or as a natural therapy for patients with excessive sleepiness. Therefore, a detailed knowledge of the medicinal effects of this widely consumed medicinal plant, could lead to a new natural option to increase the quality and quantity of wakefulness.

CONCLUSIONS
The present study demonstrates for the first time, the waking-promoting effect of yerba mate.

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