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Behavioral and Electrocorticographic Differences between Experimental Convulsive Models Triggered by Nicotine and Pilocarpine in Wistar Rats

Diferenças Comportamentais e Eletrocoticográficas Enrtre Modelos Convulsivos Experimentais Desencadeados por Nicotina e Pilocarpina em Ratos Wistar

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Abstract

The major psychoactive component in tobacco is nicotine, a stimulant that exerts its effects through the release of neurotransmitters in the central and peripheral nervous system. Few studies report nicotine toxicity as seizures. This study aimed at providing a description of behavioral and electrocortical effects caused by nicotine application (5 mg/kg i.p.) in Wistar rats and comparing the results produced by pilocarpine (300 mg/kg i.p.). Further, seizure control was assessed using different anticonvulsants. The behavior test showed six patterns that started with akinesia and evolved into tonic-clonic seizures and were compared to the convulsive model of pilocarpine that showed a difference in the latencies of the appearance of behaviors. Electrocorticographic records showed an increase in the amplitude of the tracing compatible with seizures. Although the Beta rhythm was the most powerful and consistent with other convulsive models like pilocarpine, the Theta rhythm was the predominant characteristic of the nicotine-induced seizure. The seizure control was evaluated using three agents: diazepam, phenobarbital, and scopolamine (10 mg/kg i.p.). While scopolamine was not effective in seizure control, diazepam was the most efficient drug for the attenuation of the crisis. This chemo-convulsive model should be further studied.

Keywords: Stimulant. Nicotine. Pilocarpine. Electrocorticographic. Convulsive Models.

Resumo

O principal componente psicoativo do tabaco é a nicotina, um estimulante que exerce seus efeitos por meio da liberação de neurotransmissores no sistema nervoso central e periférico. Poucos estudos relatam a toxicidade da nicotina na forma de convulsões. O objetivo deste estudo foi descrever os efeitos comportamentais e eletrocorticais causados pela aplicação de nicotina (5 mg/kg i.p.) em ratos Wistar e comparar os resultados produzidos pela pilocarpina (300 mg/kg i.p.). Além disso, o controle das crises foi avaliado com diferentes anticonvulsivantes. O teste de comportamento mostrou seis padrões que iniciaram com acinesia e evoluíram para crises tônico-clônicas e foram comparados ao modelo convulsivo da pilocarpina que apresentou diferença nas latências de aparecimento dos comportamentos. Os registros eletrocorticográficos mostraram aumento da amplitude do traçado compatível com convulsões. Embora o ritmo Beta fosse o mais potente e consistente com outros modelos convulsivos como a pilocarpina, o ritmo Theta era a característica predominante da convulsão induzida pela nicotina. O controle das crises foi avaliado usando três agentes: diazepam, fenobarbital e escopolamina (10 mg / kg i.p.). Enquanto a escopolamina não foi eficaz no controle das crises, o diazepam foi a droga mais eficaz para atenuar a crise. Este modelo quimio-convulsivo deve ser mais estudado.

Palavras-chave: *Estimulante. Nicotina. Pilocarpina. Eletrocorticográficos. Modelos Convulsivos.*

1 Introduction

Smoking is a global healthcare problem (THISTED et al., 2019). Despite decades of tobacco control policies, smoking still kills more than seven million people each year instead. It is estimated that approximately 70% of these deaths occurred in developing countries (GULIANI et al., 2019; WARREN et al., 2009). Quitting smoking significantly reduces the risk of tobacco-related morbidity and mortality, yet the addictive properties of tobacco result in high rates of relapse among smokers who try to quit. The major psychoactive component in tobacco is nicotine, a stimulant that exerts its rewarding effects through the release of dopamine and other neurotransmitters in the brain (ALLENBY et al., 2016; FERNANDO et al., 2019). With 1 billion tobacco users, nicotine is one of the most widely abused drugs in the world (BRUIJNZEEL, 2016).

Many studies have indicated that lower non-toxic doses of nicotine improve cognitive performance through modulating the release of several neurotransmitters including acetylcholine and dopamine. An interesting finding is that nicotine may be protective against the development of Parkinson's disease (BAHRAMI et al., 2017; KITAMURA et al., 2017). Several studies have also demonstrated that nicotine exposure causes cognitive deficits including impairments of visuospatial attention and impulse control, impaired working memory and other sequelae, mainly in those who are fighting against smoking abstinence (RENAUD; FOUNTAIN, 2016; VALENTINE; SOFUOGLU, 2018, 2018; HIGA et al., 2017). Indeed, even lower-level exposures associated with secondhand tobacco smoke can cause persistent neurobehavioral effects. These include increased externalizing

behavior, emotional dysfunction, and impaired neuromotor development (CAULEY et al., 2018). Furthermore, it is known that large doses of nicotine are toxic (BAHRAMI et al., 2017).

Besides that, nicotine has proconvulsive actions and, when overdosed, induces convulsive seizures in animals (IHA et al., 2017). Acute systemic injection of nicotine at high concentrations depresses locomotor activity and produces tremors, prostration, and convulsions in rats. Mice exhibiting a higher nicotine concentration in the brain suffered severe convulsions with a shorter latency than mice exhibiting a lower nicotine level after an intraperitoneal injection of nicotine (OKAMOTO et al., 1992a; FONCK et al., 2003; VALLASTER et al., 2017). However, there are few studies about its behavioral response and electrocorticographic aspects.

The present study aims to compare the behavior and electrocorticographic records of seizures caused by the acute poisoning of nicotine and pilocarpine and to evaluate the responses of anticonvulsants and antimuscarinics in the control of these seizures. We hypothesize that the nicotine has a different pattern when compared with the pilocarpine and only the anticonvulsants would control the seizure.

2 Material and Methods

2.1 Animals

For the study, seventy-two adult male Wistar rats (200g-250g) were used. All animals were kept in the Animal Research Facility of the Laboratory of Pharmacology and Toxicology of Natural Products under controlled temperature from 23ºC to 25ºC and the light-dark cycle of 12 hours, with water and food available ad libitum. All procedures followed the principles of laboratory animal care (NIH, 2011) and the guidelines proposed by Wolfensohn et al. (2013) and Lidster et al. (2016) to reduce the number and animal suffering. The experimental procedures were approved by the Council of Ethics in Experimental Animals of the Federal University of Pará (CEUA no. 4754130219).

2.2 Drugs

The anesthetics ketamine hydrochloride was purchased from Vetnil (Brazil) and the xylazine hydrochloride from Syntec (SP, Brazil). Nicotine and Pilocarpine were obtained from Sigma-Aldrich (USA). Diazepam and Phenobarbital were obtained from Cristália (Brazil). Scopolamine was purchased from Hipolabor (Brazil).

2.3 Behavioral characterization

Eighteen animals were divided into two groups (n=9): a) nicotine (5 mg/kg i.p.); b) pilocarpine (300mg/kg i.p.). Animals (n=9) received an injection and their behavior was observed for 30 minutes in a standard white acrylic box (48x38x21 cm). The latency to the displayed seizures was recorded, and behavioral changes were classified into six patterns, as suggested by Hamoy et al. (2018): 1) akinesia and motionless staring; 2) muscle relaxation with loss of posture reflex; 3) vibrissae erection; 4) flagged tail; 5) incoordination and generalized tremor, and 6) tonic-clonic seizures.

Latency of behavioral patterns observed after administration of nicotine were compared to behaviors observed after administration of pilocarpine (Table 1).

2.4 Electrocorticographic analyses

2.4.1 Experimental Design

Fifty-four animals were divided into six groups (n= 9): a) Control; b) Nicotine (5 mg/kg i.p.); c) Pilocarpine (300 mg/kg i.p.); d) Nicotine (5 mg/kg i.p.) + Diazepam (10 mg/kg i.p.); e) Nicotine (5 mg/kg i.p.) + Phenobarbital (10 mg/kg i.p.) and f) Nicotine (5 mg/kg i.p.); + Scopolamine (10 mg/kg i.p.).

2.4.2 Surgery for electrode implantation

Animals were anesthetized by ketamine hydrochloride (50 mg/kg i.p.) and xylazine hydrochloride (10 mg/kg i.p.) and positioned in a stereotaxic apparatus. Stainless steel electrodes (tip exposure 1.0 mm diameter) were placed on the dura mater above the frontal cortex at coordinates of bregma – 0.96 mm and ± 1.0 mm lateral (PAXINOS; WATSON, 2005). A screw was fixed in the occipital skull region, and the electrodes were fixed with dental acrylic cement (Methyl methacrylate monomer). The whole experiment was performed in Faraday cages and the ground electrode was fixed on the animal's right paw. The recording electrode was located on the right side of the hemisphere, and the electrode on the left side was used as a reference. All animals underwent seven days of post-implantation rest until they could be used for data collection (HAMOY et al., 2018; MELO et al., 2020; FERREIRA et al., 2020).

2.4.3 Electrocorticographic records

The methods used herein followed those described in Hamoy et al. (2018) and Melo et al. (2020). In brief, after surgery, animals were kept in individual cages. Seven days after surgery, the electrodes were connected to an analog to a data-acquisition system composed of a high impedance amplifier (Grass Technologies, P511), an oscilloscope (Protek, 6510), and a board for data acquisition and digitalization (National Instruments, Austin, TX). Data were continuously sampled at 1 kHz at a low pass of 0.3 kHz and a high pass of 0.3 Hz.

The recordings followed a standard protocol performed between 8 a.m. and 10 a.m.; 10 minutes of acclimatization of animals in the standard white acrylic box to avoid interference of records. We recorded the basal electrocorticographic activity for 5 minutes, which was used as a control treatment in the ECoG analyses. Subsequently, nicotine (5 mg/kg i.p.) or pilocarpine (300 mg/kg i.p.) was administered, and

electrocorticographic activity was additionally recorded for 30 minutes. For the evaluation of seizure control by anticonvulsants and anticholinergics, the animals first received a dose of nicotine at 5 mg/kg i.p., obeying the latency averages observed in the ECoG of 223 ± 38 seconds. After 5 minutes, the animals were submitted to one of the following agents: diazepam (BDZ - 10 mg/kg i.p.), phenobarbital (PBT - 10 mg/kg i.p.), or scopolamine (SPE - 10 mg/kg i.p.); all of them with the same dose for comparison purposes. Then, ECoG was recorded for 15 minutes. These recordings were electrically isolated and the sound and light stimuli attenuated.

2.4.4 Data analysis by checking brain power

Offline analysis was performed using software built based on the Phyton language (version 2.7). The Numpy and Scipy libraries were used for mathematical processing and the matplolib library for obtaining graphs and plots. The graphical interface was built by the PyQt4 library. The analyses were run at a frequency of up to 50 Hz, split into the Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Beta (12-28 Hz), and Gamma (28-40 Hz) bands (JALILIFAR et al., 2017) for analysis of seizure dynamics (HAMOY et al., 2018; MELO et al., 2020; FERREIRA et al., 2020).

2.4.5 Euthanasia of the animals

The rats were sacrificed using high doses of ketamine (200 mg/kg i.p.), xylazine (10 mg/kg i.p.) hydrochlorides, and diazepam (10 mg/kg i.p.) to avoid further distress, following institutional guidelines for the euthanasia of these animals.

2.5 Statistical analysis

Results were expressed as mean ± standard deviation (SD). Normality and homogeneity of variances were verified using Kolmogorov – Smirnov and Levene's tests, respectively. The seizure behavioral analyses (latencies) and electrocorticographic results were performed using one-way Analysis of variance (ANOVA) and a Tukey post-test. The minimum significance level was set at $p < 0.05$ in all cases. The GraphPad® Prism 6 software was used for all analyses.

3 Results and Discussion

The administration of 5 mg/kg of nicotine via i.p., according to Okamoto et al. (1992b), induces a series of behavioral and electrocorticographic alterations. The behavioral aspect is the most easily observed. The mean latency of the first behavioral alteration, akinesia, and motionless staring, occurred in 66.88 ± 53.89 seconds, followed by initial muscle relaxation with loss of posture reflex in 90.88 ± 61.87 seconds. Tonic-clonic seizures were observed in 490 ± 347.92 seconds. According to Turski et al. (1983), the animals are immobile in about 5-10 minutes, after the administration of pilocarpine (300 mg/kg i.p.), with subsequent display of orofacial movements, salivation, blinking, and bristling vibrissae. Tonic-clonic seizures were observed in 1400 ± 102.6 seconds. Seizures caused by nicotine had lower latencies when compared to latencies observed after pilocarpine administration (Table 1). Nicotine caused myorelaxation before the onset of seizures, this behavior was not observed after administration of pilocarpine. However, the pathways about it are unkown as the study did not evaluate the molecular aspects.

Table 1 - Behavioral patterns after nicotine (5 mg/kg i.p.) and pilocarpine (300 mg/kg i.p.) administration and latency for the occurrence of the behavioral changes. The observation period of 30 minutes

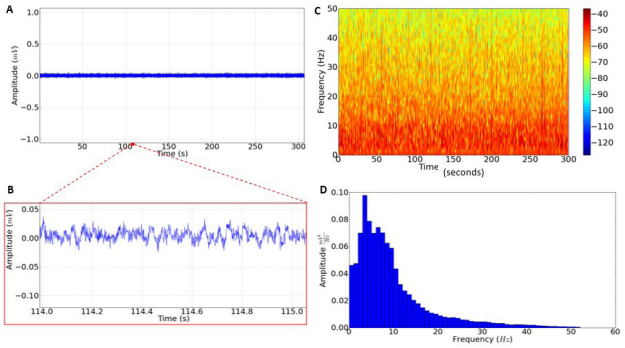
Behavior	Nicotine Latency (in seconds)	Pilocarpine Latency (in seconds)
1.Akinesia and motionless staring	66.88 ± 53.89 ***	162 ± 19.07
2.Muscle relaxation with loss of posture reflex	90.88 ± 61.87	-
3.Vibrissae erection	107.66 ± 68.09***	296 ± 40.01
4.Flagged tail	136.28 ± 89.19***	405 ± 22.11
5.Incoordination and generalized tremor	140.66 ± 83.67***	583.7 ± 57.59
6.Tonic-clonic seizures	490 ± 347.92***	1400 ± 102.6

*** After ANOVA followed by Tukey test (F = 133.9, P <0.0001) (n=9).
Source: Resource data.

With nicotine, the convulsion was observed in 140.66 ± 83.67 seconds, when the incoordination and generalized tremor arose. Besides, it also produced myorelaxation, a behavior that differs from the model with pilocarpine, as mentioned above. The six behavioral patterns used were also related to those classified in five behavior patterns by Iha et al. (2017). The akinesia and motionless staring, for example, suggest a close relationship with others models already established. However, the absence of salivation and squatting, the latter due to myorelaxation, infer a peripheral effect as depolarizing neuromuscular blocker, followed by central action and causing excitability in the same way. A similar situation was observed after intoxication by Nerium oleander (MELO et al., 2020).

The basal epidural signs of the ECoG were characterized by low amplitude oscillations (Figure 1A), with the prominent Theta (4-8 Hz) rhythm (Figure 1C) and the power of the ECoG signals concentrated in the low-frequency spectrum (Figure 1B, 1C and 1D).

Figure 1 - Basal electrocorticographic tracing (n = 9). **(A)** ECoG signal obtained from the right hemisphere using as reference the left hemisphere (5 minutes) with amplification of 1 second showing oscillations in low amplitude; **(C)** Time-frequency decomposition of a representative signal showed a higher power density at low frequencies, mainly in the Theta band (4-8 Hz); **(D)** Histogram (1 Hz per bar) displaying the energy concentration between 4-10 Hz



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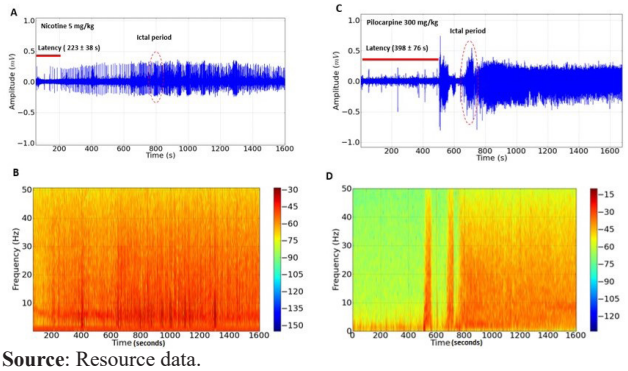
However, nicotine administration altered the rhythm of oscillations in the motor cortex of rats (Figure 2A and 2B), and the mean time for such changes in normal EcoG was 223 ± 38 seconds. The EcoG recording showed a pattern of ictal period oscillation (Figure 2A). In addition, an energy variation can be observed in the spectrogram (Figure 2B). In turn, pilocarpine as a positive control caused changes in the electrocorticographic tracing indicative of acute convulsion with a latency time of 398 ± 76 seconds, highlighting the presence of ictal period (Figure 2C) and energy distribution above 10 Hz indicated in the power spectrogram (Figure 2D).

Figure 2 - Epidural signal after the triggering of the displayed seizure (1600 seconds) (n = 9). (A) The electrocorticographic tracings (ECoG) shown an increase in the amplitude of oscillations, with the display of nicotine latency period and ictal period; **(B)** Time-frequency decomposition of the seizure, highlighting the large increase in power between 0-50 Hz during the nicotine ictal period; **(C)** The electrocorticographic tracings (ECoG) shown an increase in the amplitude of oscillations, with the display of pilocarpine latency period and ictal period; **(D)** Energy distribution spectrogram after application of pilocarpine. The red line indicates the latency to change the ECoG record. The red dotted circle represents the ictal period



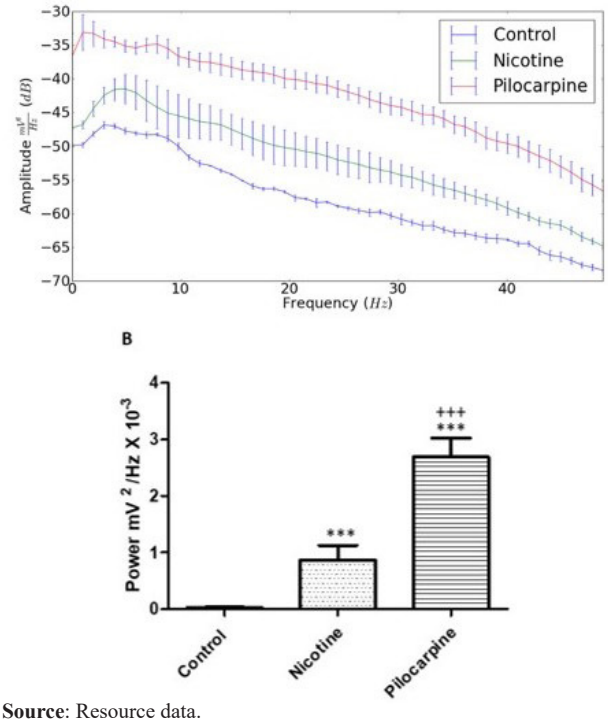
Source: Resource data.

Another point that deserves to be highlighted is the dose necessary for the production of the seizure and its electrophysiological effects. According to what was predicted and made by Okamoto et al. (1992a, 1992b), 5 mg/kg via i.p. of nicotine produced alterations up to 6º pattern (tonic-clonic seizure) of behavioral characteristics of the animals. No deaths were recorded during the experiment. Moreover, the nicotine dose mentioned above was used to ensure that 100% of the animals will display the seizure up to 6º pattern of behavioral characterization. Iha et al. (2017) conducted studies with a lower dose, however, the animals did not evolve to tonic-clonic seizures. Miner and Collins (1989) demonstrated nicotine-induced seizures with 2 mg/kg to 7 mg/kg and indicated a difference between the routes of administration. While intravenous has a small latency and lower doses can be used, i.p. presented higher latencies and needed high doses to display the seizure.

It is important to emphasize that seizures are classically characterized as hypersynchronous activation of large neuronal populations (FISHER et al., 2017). As seen with the behavioral characterization, the high dose administration of nicotine induces convulsion with differences about pilocarpine, like the muscle relaxation. The latency period at 223 ± 38 seconds, the shots that increase in amplitude and frequency are remarkable and relatively short in duration, which constitutes a pre-ictal period with significant changes in the spectrogram of energy distribution and allows full recovery of the animal. This find is in accordance with the postulate by Easter et al. (2009). The ECoG recording showed a pattern of oscillation of the ictal period, which may characterize a seizure similar to pilocarpine (NIEDERMEYER, 2001; PEDLEY et al., 2003). Thus, it is possible to establish the existence of cycles that repeat and are separated by interictal periods with oscillation of amplitude and frequency of the shots, as Fonck et al. (2003) said about the intervals characterized by little or no abnormal electrical activity. On the contrary, pilocarpine has the presence of a latent period followed by the appearance of spontaneous recurrent seizures (CAVALHEIRO et al., 1991).

In the power spectral density (PSD) graph, an increase in power was demonstrated at frequencies of 0-50 Hz after nicotine administration, when compared to the control. However, after pilocarpine administration, the power distribution was higher than that caused by nicotine administration (Figure 3A). This information can be verified after the power analysis, linearly, at the frequencies of 0-50 Hz, where the control presented an average of 0.02527 ± 0.008474 mV^2/Hz $x10^{-3}$ and demonstrated statistical difference to the group that received nicotine with an average of 0.8604 ± 0.2677 mV^2/Hz $x10^{-3}$. In addition, the nicotine group also showed a statistical difference for the pilocarpine group, which showed higher potency (2.689 ± 0.3368 mV^2/Hz $x10^{-3}$) ($p< 0.0001$; $F= 270.7$) (Figure 3B).

Figure 3 - (A) Spectral power distribution of the control, nicotine, and pilocarpine groups. **(B)** Evaluation of the powers on a linear scale of the respective groups. (***) statistical difference to the control; (+++) statistical difference to the nicotine group. (p <0.0001, n = 9) using one-way ANOVA, followed by a Tukey test



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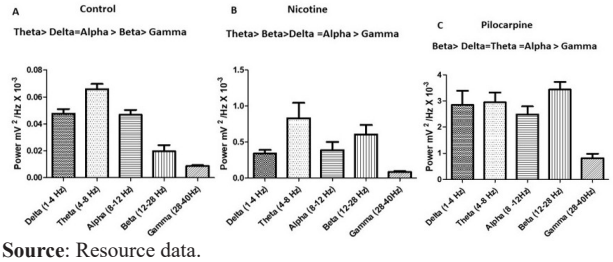
It is also important to highlight the basal spectral decomposition, which showed a concentration of power in low frequencies, with greater activity in Theta oscillations. The dominant powers were recorded as follows: **Theta > Delta = Alpha > Beta > Gamma** (p< 0.0001; F= 386.1) (Figure 4A). Frequency amplitudes up to 40 Hz increased during the ictal period in seizures induced by nicotine and pilocarpine. During the period of convulsion of the nicotine group, more intense oscillations were obtained, and the Theta and Beta band reached the highest amplitudes among the other frequencies, as follows: Theta > Beta > Delta = Alpha > Gamma (p< 0.0001; F= 69.3) (Figure 4B). For pilocarpine-induced seizures, there were changes in the predominance of power in the Beta band, maintaining the prevalence of oscillations as follows: Beta > Delta = Theta = Alpha > Gamma (p< .0001; F= 45.66) (Figure 4C).

Figure 4 - Average power of Delta, Theta, Alpha, Beta, and Gamma during registrations. (A) Oscillations in the control group; **(B)** Oscillations in the nicotine group; **(C)** Oscillations of the pilocarpine group. (p <0.0001, n = 9) using one-way ANOVA, followed by a Tukey test



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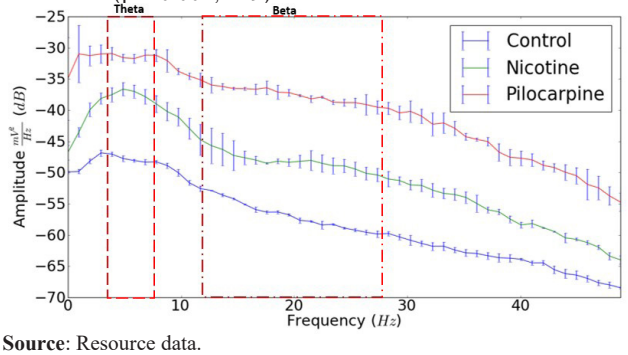
When it compares pilocarpine to nicotine, it is noted that the former has a higher potency distribution than the latter by about 3 times. Pilocarpine produces convulsive activity with a noticeable ictal period in the ECoG, which, according to Cavalheiro (1995), correlates well with the sequence of behavioral alterations that can be observed. As we can see in the ECoG and the energy distribution spectrogram, normal background activity was replaced by rapid activity, still with the prominence of the Theta rhythm. However, subsequently, the rapid activity of high amplitude is superimposed on this waveband, and low amplitude spicules are recorded. Typical electrocorticographic seizures are then observed after the high amplitude and frequency peak, with emphasis on the Beta rhythm (TURSKI et al., 1983).

During the recordings, were identified ictal period with the presence of potential burst, in which the level of energy captured by the electrode increases. Thus, the PSD was analyzed during the potential burst, to examine the predominant powers for nicotine in comparison with the chemoconvulsant model of pilocarpine (Figure 5). PSD demonstrated increased Theta and Beta power for nicotine.

Figure 5 - Energy distribution graph, during the potential burst in the ictal period, demonstrates that the prevalence of potency was maintained in the Theta and Beta bands, based on power increase calculation (p < 0.001, n=9)



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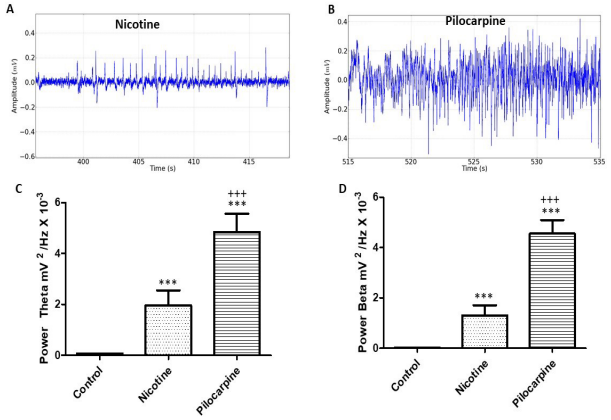
During the tracing in the potential burst, in the linear analysis of the Theta power, the control group presented an average of 0.06570 ± 0.003945 mV^2/Hz $x10^{-3}$, maintaining statistical difference to the nicotine group (1.965 ± 0.5878 mV^2/Hz $x10^{-3}$) (Figure 6A and 6B). However, the highest potency in the Theta band was observed for the group receiving pilocarpine (4.847 ± 0.7146 mV^2/Hz $x10^{-3}$), presenting differences to the basal and nicotine group (p<0.0001; F= 182.8) (Figure 6C). For the Beta band, the power of the control group presented an average of 0.01969 ± 0.004569 mV^2/Hz $x10^{-3}$, with a statistical difference to the nicotine group (1.302 ± 0.400 mV^2/Hz $x10^{-3}$), and the power of this band increased the most proportionally concerning the other powers, mainly in the pilocarpine group, which presented an average of 4.553 ± 0.54115 mV^2/Hz $x10^{-3}$ and maintained statistical difference for the control and nicotine groups (p<0.0001; F= 325.3) (Figure 6D).

Figure 6 - (A) Electrocorticographic tracing during the potential burst after nicotine administration; **(B)** ECoG recording during the pilocarpine-induced potential burst; **(C)** Evaluation of linear power for Theta band during the potential burst; **(D)** Linear analysis of the Beta band during the potential burst. (***) Statistical difference to the control; (+++) statistical difference to the nicotine group. (p <0.0001, n = 9) using one-way ANOVA, followed by a Tukey test



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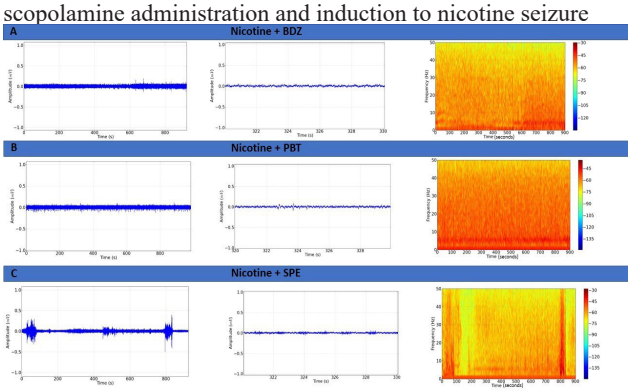
Grasse et al. (2013) reported a prolonged synchronization of Theta rhythm before the development of spontaneous seizures in the hippocampus. So, it may be a key factor in the recruitment of neuronal networks for the triggering of the displayed seizure, especially in the case of nicotine, in which this band was predominant - compared to the other bands up to 50 Hz - during the ictal period and the potential burst. This wave is present in various types of activity of the locomotor system, sometimes occurring in firing caused by harmful stimuli. In addition, Theta oscillation functions as a computational process that gathers the activity of sensory neurons and memory neurons that, when activated, affect behavior (BUZSÁKI, 2002). This rhythm is established when the activities are completed in routine, with the automaticity of repetitive tasks (DESAI et al., 2015). Thetas waves are also attributed to physiological conditions, such as the connection in separating the past, present, and future to establish memory (BUZSÁKI; MOSER, 2013) and being prominent when there is a state of anxiety relief (SUETSUGI et al., 2000). Thus, this possibly explains the existence of repetitive cycles of ictal periods with high neuronal synchronization, in addition to the harmful character of this substance in humans.

About nicotine, it is worth emphasizing that Beta strength is the second-highest in potency during the presence of potential burst in the ictal period. However, in the pilocarpine group, Beta strength is the predominant force under the same situations. Little and Brown (2014) state that Beta oscillations play a fundamental role in physiological motor functioning by controlling the decoding capacity of information, which is limited when the activity of this waveband is high, favoring pathological conditions such as Parkinson's disease. In addition, Beta waves are also associated with alertness, focus, and active thinking (KIM et al., 2013). This rhythm was the one that increased the most - proportionally - with the other rhythms, both in the nicotine group and pilocarpine group. In the latter group, beta power was about three times larger than the comparative focus of this study (Figure 4). The other bands have significance in the research. Still, attention is given to Theta and Beta because they configure the prevalence of potency during the potential burst of the ictal period. Besides, Beta is the predominant band in other convulsive models, such as cunaniol and pentylenetetrazole (HAMOY et al., 2018). Thus, despite the prominence of Theta in nicotine, the follow-up of neuronal synchronization according to other convulsive substances is observed. In the analysis of the potential burst, it is possible to verify the greater amplitude and frequency, signaling higher potency of this hyper-synchronization of pilocarpine than nicotine (Figures 5 and 6).

It was observed with the electrocorticographic tracing, after use of diazepam, that there were no outbreaks of nicotine-induced seizures, as demonstrated in the enlargement of the tracing and the energy distribution spectrogram (Figure 7A). Phenobarbital was the second drug used for the test and was also effective in controlling nicotine-triggered seizures (Figure 7B). To investigate the mechanism by which seizures occur and to relate to seizures caused by pilocarpine, scopolamine was used to prevent the outbreak of nicotine-induced seizures, without success (Figure 7C).

Figure 7 - Evaluation of anticonvulsant activity in the control of the deflagration of nicotine-induced seizures. **(A)** Electrocorticographic tracing with 900 seconds duration after application of diazepam (BDZ) and induction of seizures with nicotine, with enlargement of the tracing and energy distribution spectrogram; **(B)** ECoG registration after phenobarbital (PBT) application, followed by nicotine convulsive dose application, as previously explained; **(C)** Electroencephalographic tracing after scopolamine administration and induction to nicotine seizure



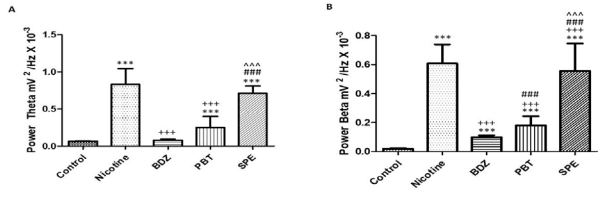
Source: Resource data.

Scopolamine had no action in the control of the seizure triggered by the nicotine, agreeing with Turski et al. (1989), who indicates that the use of it in the cases of poisoning by pilocarpine did not influence the development of the convulsive condition too. These analyses were confirmed by the records in the ECoG (and expansion of the obtained tracing) along with the energy spectrogram, signaling an activation mechanism different from the pilocarpine. Thus, it opens the way for future studies that investigate the mechanism of seizures induced by acute nicotine poisoning

and new antiepileptic drugs useful for this situation.

In other when it considered the prevailing power during the convulsive condition caused by nicotine, for the power in the frequency range in Theta, the control group presented an average of 0.06570 ± 0.003945 mV^2/Hz $x10^{-3}$, showing no statistical difference to the group receiving diazepam (0.07962 ± 0.01356 mV^2/Hz $x10^{-3}$). The phenobarbital group demonstrated the reduction of Theta with an average of 0.2496 ± 0.1497 mV^2/Hz $x10^{-3}$, maintaining statistical difference to the nicotine (0.8307 ± 0.2138 mV^2/Hz $x10^{-3}$) and control group. Thus, phenobarbital was shown to have lower efficacy than diazepam in controlling the growth of Theta strength. Also, for the group treated with scopolamine (0.7108 ± 0.09794 mV^2/Hz $x10^{-3}$), there was no statistical difference to the nicotine group ($p<0.0001$; $F=74.86$) (Figure 8A).

Figure 8 - Evaluation of anticonvulsant activity in the decrease of the main powers that increase during the nicotine-induced seizure. (**A)** Evaluation of anticonvulsant action in reduction of Theta strength; **(B)** Analysis of anticonvulsant activity for Beta strength. (***) Statistical difference to the control; (+++) Statistical difference to the nicotine group; (###) Difference for the BDZ group, (^^^) indicates difference for the PBT group. ($p<0.0001$, $n = 9$) using one-way ANOVA, followed by a Tukey test.



Source: Resource data.

In turn, for the powers in the Beta band, the control group had an average of 0.01969 ± 0.004569 mV^2/Hz $x10^{-3}$, which showed a statistical difference to the diazepam (0.09910 ± 0.01319 mV^2/Hz $x10^{-3}$), phenobarbital (0.1810 ± 0.06338 mV^2/Hz $x10^{-3}$), and scopolamine (0.5551 ± 0.1902 mV^2/Hz $x10^{-3}$) groups. This exposes the reduction of the frequency of the Beta band in the presence of diazepam and phenobarbital anticonvulsants, but it does not return to the basal state. The scopolamine group showed no statistical difference to the nicotine group (0.6061 ± 0.1325 mV^2/Hz $x10^{-3}$), revealing inefficacy in the control of seizures ($p<0.0001$; $F=56.61$) (Figure 8B).

The predominant powers during the convulsive condition caused by nicotine were Theta and Beta. In the case of the Theta band, with diazepam, it returned to levels similar to that of the basal state. With phenobarbital, despite a significant reduction in this wave rate and control of the convulsive condition, it did not achieve the same effectiveness as a benzodiazepine. In turn, scopolamine showed no statistical difference to the group treated only with nicotine, emphasizing its inefficient character in controlling the seizures induced by this substance. When the potency in the Beta band is analyzed, there was an effective control by diazepam and phenobarbital but without return to statistical levels close to the baseline. Again, scopolamine was not efficient, reinforcing the possible difference in the mechanism of triggering seizures between nicotine and pilocarpine. Thus, what was previously postulated by the ECoG is confirmed. Agreeing to Hamoy et al. (2018), who demonstrated the convulsion triggered by Cunaniol, diazepam was the drug that best-controlled seizures (Figure 8).

3 Conclusion

The analysis confirms the efficacy of antiepileptic drugs showed that diazepam is the best choice, followed by Phenobarbital. Scopolamine did not have control as expected. That chemoconvulsive model is different when compared to pilocarpine and needs to be more studied mainly because it's a compound present in an extremely distributed licit drug.

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