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Research report

EEG dissociation induced by muscarinic receptor antagonists: Coherent 40 Hz oscillations in a background of slow waves and spindles

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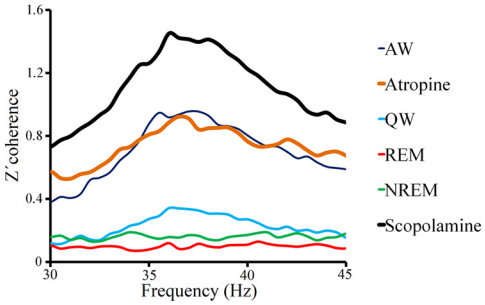
GRAPHICAL ABSTRACT

Gamma coherence is high following Atropine or Scopolamine administration



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ABSTRACT

Mesopontine and basal forebrain cholinergic neurons are involved in the control of behavioral states and cognitive functions. Animals treated with cholinergic muscarinic receptor antagonists display a dissociated state characterized by behavioral wakefulness (W) associated with high amplitude slow oscillations and spindles in the electroencephalogram (EEG), similar to those that occur during non-REM (NREM) sleep.

Oscillations in the gamma frequency band (≈ 40 Hz) of the EEG also play a critical role during W and cognition. Hence, the present study was conducted to determine the effect of muscarinic antagonists on the EEG gamma band power and coherence.

Five cats were implanted with electrodes in different cortices to monitor the EEG. The effects of atropine and scopolamine on power and coherence within the low gamma frequency band (30–45 Hz) from pairs of EEG recordings were analyzed and compared to gamma activity during sleep and W.

Muscarinic antagonists induced a NREM sleep-like EEG profile that was accompanied by a large increase in gamma power and coherence. The values of gamma coherence were similar to that occurring during alert W (AW), and greater than in quiet W, NREM and REM sleep.

We conclude that under atropine or scopolamine, functional interactions between cortical areas in the gamma frequency band remain high, as they are during AW. This significant functional connectivity at high frequency may explain why the animals remain awake in spite of the presence of slow waves and spindles.

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1. Introduction

Mesopontine and basal forebrain cholinergic neurons are critically involved in the generation and maintenance of wakefulness (W) and rapid-eye-movements (REM) sleep [1,2]. In this regard, animals treated with atropine, a muscarinic receptor antagonist, display high voltage slow waves and spindles in the electroencephalogram (EEG) that resembles non-rapid eyes movement (NREM) sleep; however, they remain behaviorally awake and active [3]. Furthermore, atropine decreases the electrocortical arousal response elicited either by sensory or midbrain reticular formation stimulation, but the gross behavior in response to such stimuli is not affected [4,5]. This “dissociation” in which waking behavior coexists with NREM sleep-like EEG was observed in cats, dogs, rats and rabbits [3,6–9].

The brain integrates fragmentary neural events that occur at different times and locations into a unified perceptual experience. EEG oscillations in the gamma frequency band (mainly around 40 Hz) are involved in the integration or binding of spatially separated but temporally correlated neural events [10,11]. An increase in gamma power typically appears during behaviors that are characterized by cognitive processing of external percepts or internally generated thoughts and images [12–14]. High gamma power has been observed during attentive W not only in humans, but also in animals [15–19]. Furthermore, gamma coherence between different brain areas have been viewed as a possible neural correlate of consciousness [20,21]; the degree of EEG coherence between two cortical regions is believed to reflect the strength of the functional interconnections that occur between them [22,23].

In the cat, EEG "bursts" of 35–40 Hz oscillations of 200–500 ms and approximately 25 μV are readily observed in raw EEG recordings during alert wakefulness (AW) [24]. The EEG coherence in this frequency band is greater during AW than quiet wakefulness (QW); it decreases to a lower level during NREM sleep, and reaches its nadir during REM sleep [24–26]. Additionally, high gamma coherence has been observed during cataplexy induced by microinjections of carbachol into the nucleus pontis oralis (when the animal is fully awake but with complete muscle atonia), but not during REM sleep induced by the same procedure [27].

In order to find clues that account for the lack of correspondence between gross behavior and EEG patterns induced by muscarinic receptor antagonists, in the present report we studied the power and coherence in the EEG low gamma frequency band (30–45 Hz) of the cat treated with atropine and scopolamine, two commonly employed non-selective competitive muscarinic antagonists. We compared these results with the EEG gamma power and coherence that is present during naturally-occurring W and sleep.

2. Material and methods

Five adult cats were used in this study. The animals were obtained from the Institutional Animal Care Facility and determined to be in good health by veterinarians of the institution. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th edition, National Academy Press, Washington DC, 2011) and were approved by the Institutional Animal Care Commission. Adequate measures were taken to minimize pain, discomfort or stress of the animals. In addition, all efforts were made to use the minimum number of animals necessary to produce reliable scientific data.

The animals were also employed in previous studies [24,25,27]. These 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

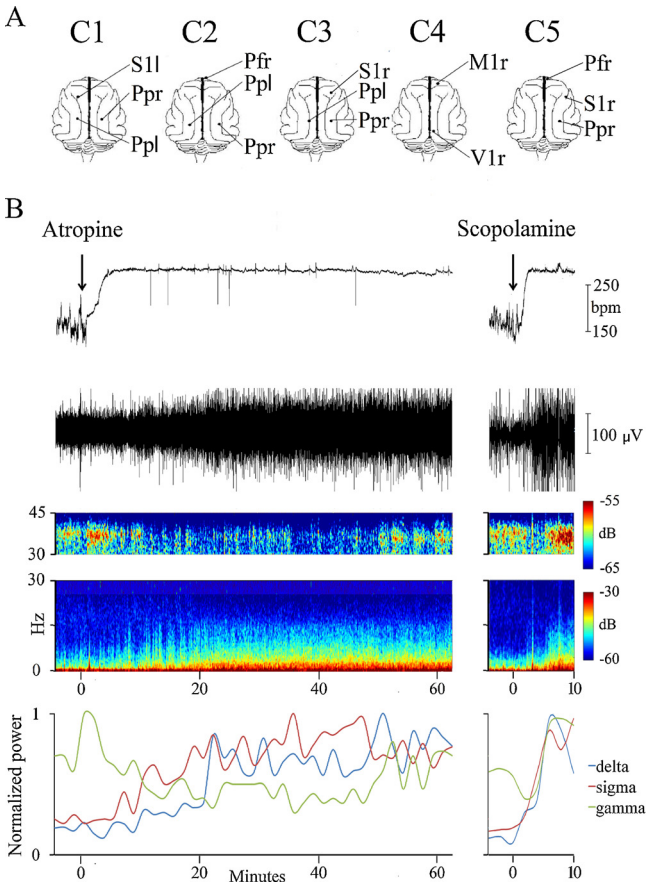

Fig. 1. A. Position of the recording electrodes. Recordings from these electrodes were referred to a common referential electrode, which was located over the frontal sinus. C1–C5, individual animals. M1, primary motor cortex; Pf, prefrontal cortex; Pp, posterior-parietal cortex; S1, somato-sensory primary cortex; V1, visual primary cortex; r, right; l, left. B. The tachogram, the EEG of the posterior parietal cortex, and its spectrogram (0–30 Hz and 30–45 Hz have different power scale) are shown following atropine or scopolamine injections (arrows). Delta, sigma and gamma normalized power are also shown. bpm, beats per minute; EEG, electroencephalogram.

head was positioned in a stereotaxic frame and the skull was exposed. Stainless steel screw electrodes (1.4 mm diameter) were placed on the surface (above the dura matter) of different cortical areas. Fig. 1A shows the sites of the recording electrodes whose signals were analyzed in the present study. The electrodes were connected to a Winchester plug, which together with two plastic tubes (used to fix the animal's head position without pain or pressure) were bonded to the skull with acrylic cement. After the animals recovered from the preceding surgical procedures (that usually takes three weeks), they were adapted to the recording environment for a period of at least two weeks.

Experimental sessions of 4 h were conducted between 11 A.M. and 3 P.M. in a temperature-controlled environment (21–23 °C). During these sessions (as well as during the adaptation sessions), the animal's head was held in a stereotaxic position by four steel bars that were placed into the chronically-implanted plastic tubes, while the body rested in a sleeping bag.

The simultaneous activity of 3 cortical areas (two in C4) was recorded with a monopolar (referential) configuration, utilizing a common reference electrode located in the left frontal sinus. The electromyogram (EMG) of the nuchal muscles, which was recorded by means of acutely placed bipolar electrode, was also monitored. The electrocardiogram (ECG), by electrodes acutely placed on the skin over the pre-cordial region, and respiratory activity by means of a micro-effort piezo-crystal infant sensor, were also recorded [28]. Each cat was

recorded daily for approximately 30 days in order to obtain complete basal and treatment data sets.

Bioelectric signals were amplified (×1000), filtered (0.1–500 Hz), sampled (1024 Hz, 2^{16} bits) and stored in a PC using the Spike 2 software (Cambridge Electronic Design).

Data were obtained after atropine or scopolamine administration as well as during spontaneously-occurring QW, NREM sleep and REM sleep. AW was induced for a period of 300 s by a sound stimulus, which was introduced approximately 30 min after the beginning of the recording session. The sound stimulus consisted of clicks (0.1 ms in duration) of 60–80 dB SPL in intensity with a variable frequency of presentation (1–500 Hz, modified at random) in order to avoid habituation [24,27,29]. Atropine (0.2 and 0.4 mg/kg s/c, Sigma) or scopolamine (1 mg/kg s/c., Sigma) were administrated in 4 experimental sessions to 5 and 3 animals, respectively; the doses were similar than in previous studies [30–32]. In pilot experiments where vehicle (saline) was administered, the EEG analysis during W and sleep showed results similar to those of the baseline condition; therefore, baseline experiments (without saline administration) were used for the analysis.

Data was analyzed as in our previous studies [24,25,27]. Sleep and W were quantified in epochs of 10 s. In order to analyze gamma coherence between pairs of EEG electrodes, 12 artifact-free periods of 100 s were examined during each behavioral state (1200 s for each behavioral state). The data of the drug administration experiments were obtained in four recording sessions for each drug and dose. Quantitative analyses were performed in time windows between 50 and 60 min following atropine, and between 5 and 15 min following scopolamine administration; these time periods correspond with the peaks of the EEG effects induced by these drugs (Fig. 1B). Furthermore, control data were obtained from four recording sessions during AW, QW, NREM and REM sleep.

For each selected period of 100 s, the Magnitude Squared Coherence was analyzed by means of Spike 2 script COHER 1S (Cambridge Electronic Design) (see Castro et al. 2013, for details in the definition of coherence). Coherence between two EEG channels that were recorded simultaneously during 100 s periods was analyzed. This period of analysis was divided into 100 time-blocks with a sample rate of 1024 Hz, a bin size of 2048 samples and a resolution of 0.5 Hz. The random level of coherence was approximately 0.1 [24]. In order to normalize the data and conduct parametric statistical tests, the Fisher z' transform to the gamma coherence values was utilized. In order to process the power spectrum of the EEG (by means of the Spike 2 script COHER 1S), we employed the same time-windows as for the coherence analysis.

Recordings were also filtered (band pass 30–45 Hz) using Spike 2 digital finite impulse response filters. The amplitude of simultaneously recorded pairs of filtered EEG signals was also analyzed by means of auto-correlation (ACF) cross-correlation (CCF) functions. Averages, and spectrograms were also performed. In addition, the RR intervals of the ECG signal was also analyzed and plotted against time (tachogram) [28,33] (see Fig. 1B).

In Figs. 1 and 4, two different approaches were used in order to estimate EEG power. In Fig. 1 a multitaper method was used as described by [34]. This method utilized a series of discrete prolate spheroidal sequences (Slepian) for the Fast Fourier Transform. The procedure reduces the variance of the power spectrum estimate, offering a better power estimation. In Fig. 4 wavelet transform was applied in order to improve time and frequency localization. We used Morlet wavelet because of its proven suitability for EEG analysis [35]. Both analyses were performed employing Chronux and ND Toolbox running on self-built MATLAB routines.

Power and z'-coherence of the gamma band for each EEG channel or derivative (pair of EEG channels) were also averaged across behavioral states and drug treatments. Data were expressed as the mean ± standard error. The significance of the differences among behavioral states was evaluated for each cat with one-way ANOVA and Tamhane test. Because the electrodes positions were not the same in all the animals (Fig. 1A), in order to analyze the effect of the drugs in the whole group of animals, the mean intra-hemispheric z'-coherence of the gamma band between anterior (S1 for C1 and C2, Pf for C3 and C5, and M1 for C4; see Fig. 1A) and posterior (V1 for C4, and Pp for the rest of the animals) cortices was evaluated (Fig. 6). For this purpose, we utilized the repeated measures ANOVA (rmANOVA) and Bonferroni *post hoc* test. The criterion used to reject null hypotheses was $p < 0.05$.

3. Results

3.1. Behavior

Following atropine (0.2 and 0.4 mg/kg) or scopolamine (0.1 mg/kg) administration, the animals were awake and were able to track the experimenters with their eyes; they were also capable of vocalization. When the animals, still under the effects of the drugs, were reintroduced to freely-moving condition (at the end of the experiments, 3–4 h after drug injection), they were able to ambulate.

3.2. Analysis of the EEG recordings

As expected, both doses of atropine increased heart rate and eliminated its variability (an example is shown in the tachogram of Fig. 1B); however, the agent produced clear central effects only at 0.4 mg/kg. Hence, we only analyzed the effects of the higher dose.

Atropine (0.4 mg/kg) produced high amplitude slow waves and spindles; i.e., a NREM sleep-like EEG (Fig. 1B); however, the animals continued in behavioral W. Cardiovascular effects reached their greatest values with a latency of 3–5 min, whereas EEG slow waves and spindles were fully developed after 30–40 min. These electrographic events are readily observed in the EEG and spectrogram of Fig. 1B.

In contrast to NREM sleep, but similar to AW, intense gamma activity (30–45 Hz) was present under atropine. In the spectrogram of Fig. 1B, following the atropine injection there was an intense gamma activity due to the high alertness induced by the puncture. Thereafter, there was a relative decrease in gamma power (to the level of QW), and after 50 min, gamma power was very high again. This increase in gamma power was accompanied by an increase in delta (0.5–3 Hz) and sigma (11–14 Hz) power (Fig. 1B).

The heart rate and EEG effect induced by scopolamine is also shown in Fig. 1B. The main difference with atropine is that the latency of the EEG effects is only about 3 min.

In raw recordings, it can be readily observed that gamma activity following atropine administration was present in the form of gamma "bursts", as was previously described for AW [24,25,27] (Fig. 2); these gamma “burst” seemed to be coupled between cortices. Similar results were obtained following scopolamine administration (Fig. 3). In 30–45 Hz filtered (band pass) recordings, it is readily apparent that after scopolamine, gamma activity and gamma coupling between cortices is very similar to during AW, but different compared to NREM sleep (Fig. 3). On the contrary, under scopolamine, slow waves and spindles were very similar compared to NREM sleep.

In summary, following either atropine or scopolamine administration, the EEG consisted of coupled gamma oscillations in a background of slow waves and spindles. Because these NREM sleep-like phenomena (delta waves and spindles) have been already described [36], we did not analyze them in detail.

3.3. Characterization of the gamma “bursts” following atropine/scopolamine treatment

The characteristics of the gamma “bursts” present following muscarinic antagonist treatment were analyzed. The gamma activity during these conditions was compared with the activity present in AW and NREM sleep. Because (disregarding the latency, Fig. 1B) the central

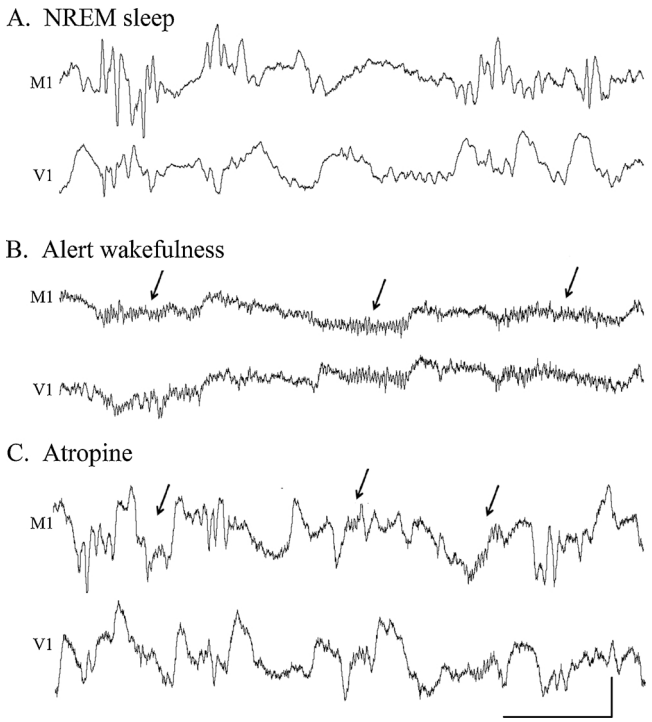

Fig. 2. Effects of atropine. Simultaneous raw EEG recordings from the primary motor cortex (M1) and primary visual cortex (V1) during: NREM sleep (A), alert wakefulness (B) and atropine administration (C). Arrows indicate gamma "burst" oscillations. Calibration bars: 1 s and 200 μV.

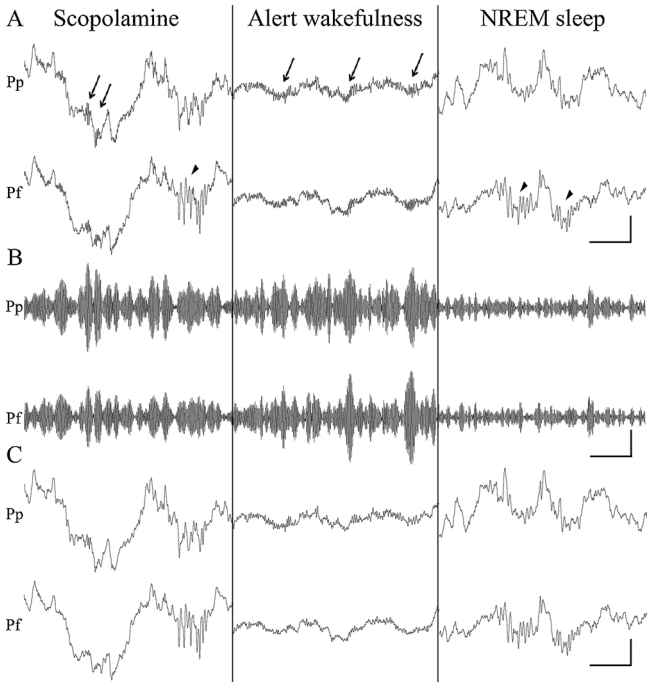

Fig. 3. Effects of scopolamine. The activity of the prefrontal (Pf) and posterior parietal cortex (Pp) during NREM sleep, alert wakefulness and following scopolamine administration are shown in: (A) simultaneous raw recordings, (B) 30–45 Hz and (C) 0.5–30 Hz band-pass filtered EEG recordings. Arrows and arrowheads indicate gamma "burst" and sleep spindles, respectively. Calibration bars: 1 s, 200 μV for A and C; 20 μV for B.

effects of scopolamine and atropine were similar, in this section we will do an overall description of gamma "bursts" under the effect of both muscarinic antagonists.

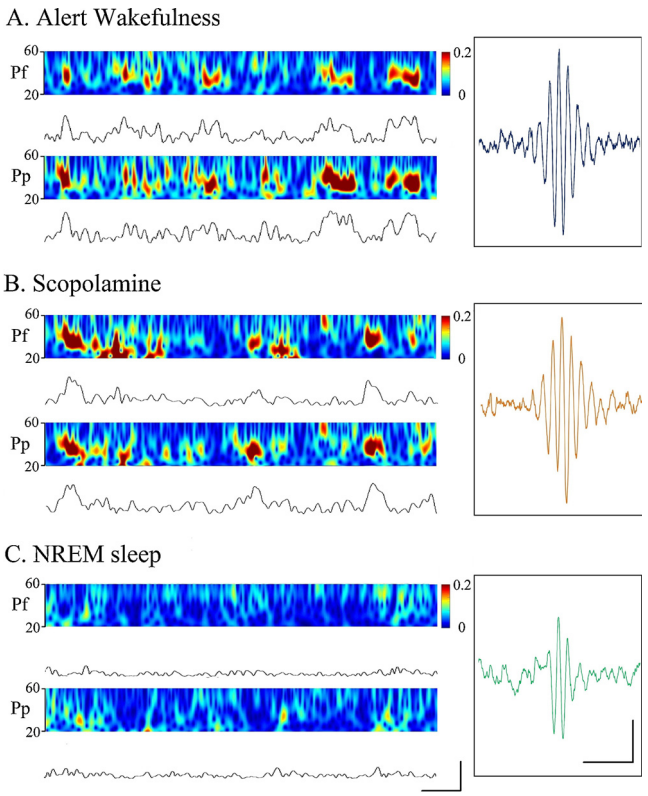

Fig. 4. Spectrograms (by means of wavelet function) and rectified gamma band (30–45 Hz) or gamma envelopes, during AW (A), under scopolamine (B) and during NREM sleep (C). For the spectrograms, frequency is represented in the ordinates, while the color code shows a wavelet coefficient that represent in relative units the energy of the signal. Calibration bars: 400 ms and 30 μV (for the envelopes). Insets. Average gamma "bursts" from a selected and filtered (high pass 3 Hz) recording of the prefrontal cortex. 100 random bursts were selected and averaged; the trigger was the peak of the higher amplitude wave of the "burst". Calibration bars: 10 μV and 200 ms.

In Fig. 4, gamma spectrograms and gamma envelopes were used to compare the dynamics of the "bursts" among AW, scopolamine and NREM sleep. While there is a marked coupling between cortices during AW and following scopolamine, this coupling is decreased during NREM sleep. The gamma burst waveforms were also similar (both in amplitude and duration) during AW and following muscarinic antagonists' treatment (Fig. 4, insets). Dominant frequency analysis revealed not significant differences between AW, atropine, and NREM sleep (37 ± 2 Hz, 36 ± 2 Hz and 35 ± 2 Hz, respectively; ANOVA). During NREM sleep although the dominant frequency is the same, Fig. 4 clearly shows that NREM sleep bursts have lower amplitude and duration.

Fig. 5A shows examples of the ACF of the gamma burst during AW and following atropine administration in the prefrontal cortex of a representative animal. CCF functions between filtered recordings (30–45 Hz) of prefrontal and posterior parietal cortices during AW and following atropine are also shown. ACFs and CCFs were similar during AW and following this pharmacological treatment.

In Fig. 5B the average of gamma bursts was shown. The gamma "bursts" under scopolamine (as well as under atropine, not shown) were associated with the slow waves negativity; similar association with the slow waves can be observed during NREM sleep.

3.4. Gamma power and coherence

The power spectrum analysis of the EEG on a representative animal and cortical site is shown in Fig. 6A. Power is similar in the recordings during NREM sleep, atropine and scopolamine for frequencies under

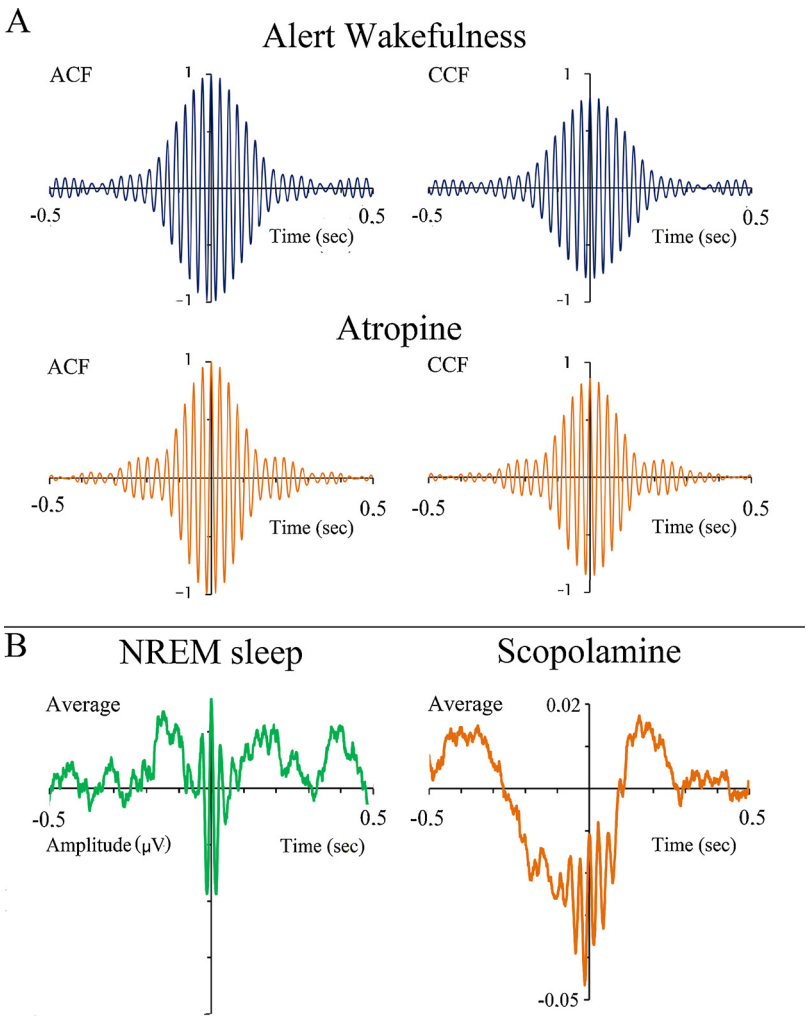

Fig. 5. A. Autocorrelation (ACF) and cross-correlation functions (CCF) from filtered (30–45 Hz) recordings. The functions were processed from 300 s EEG recording windows of the prefrontal cortex (ACF) and from simultaneous EEG recordings from the prefrontal cortex and posterior parietal cortex (CCF). The ACF and CCF are shown during alert wakefulness and following atropine administration. B. Averaged gamma "burst" of a selected raw recording of the prefrontal cortex. 100 random bursts were selected and averaged; the trigger was the peak of the higher amplitude wave of the "burst". The averages are shown during NREM sleep and following scopolamine administration.

30 Hz. In contrast, gamma band (30–45 Hz) power is larger following atropine and scopolamine administration compared to that present during NREM sleep (Fig. 6A, inset). Tables 1 and 2 present the results of a statistical analysis of gamma power for all animals (and recorded cortices). Gamma power under atropine (Table 1) or scopolamine (Table 2) was greater than during NREM sleep for all animals and most of the cortices (Table 1).

The coherence function was utilized to conduct an in-depth analysis of the 'coupling' between the gamma oscillations that were simultaneously recorded in different cortices during W and sleep, as well as under the effect of atropine and scopolamine. The z'-coherence profiles for a representative pair of EEG leads of one cat, of twelve 100-second periods and their average for AW as well as for atropine and scopolamine-induced states are exhibited in Fig. 6B1 and B2, respectively. z'-coherence profiles under atropine were similar to those during AW whereas following scopolamine, the z'-coherence profiles were still greater. The z-coherence average profiles for the different physiological and drug-induced states are displayed in Fig. 6C.

Fig. 7 shows the z-coherence between the "anterior" and "posterior" cortices for all animals treated with atropine. Following atropine administration, gamma coherence was larger than during QW, NREM and REM sleep.

Statistical analyses for z´-coherence are also shown for each animal (and cortical pairs) treated with atropine and scopolamine (Tables 3 and 4). In all the cats and derivatives analyzed, z'-coherence under atropine or scopolamine was greater than during NREM and REM sleep. Depending of the cat or derivative the z-coherence values were similar to AW (in most of the cases) or to QW.

4. Discussion

It is well known that muscarinic receptor antagonists induce behavioral W associated with slow waves and sleep spindles in the EEG (the EEG markers of NREM sleep). In the present report, we demonstrated that under the effect of atropine or scopolamine, coherent gamma (≈ 40 Hz) oscillations are also conspicuous.

Coherent gamma oscillations are a distinctive characteristic of attentive or alert W in the cat [24–27]. Therefore, this new finding has two important conceptual insights. First, acetylcholine, acting through muscarinic receptors, is not necessary to generate neocortical 40-Hz EEG oscillations. Second, the large and coherent gamma oscillations are likely the electrocortical footprints of the behavioral W that is present under the muscarinic cholinergic antagonists' treatment. In other words, this "dissociated" EEG with slow waves and sleep spindles, but also with coherent 40 Hz-oscillations (a trait of AW), may be the neurophysiologic basis of the "classic" EEG and behavior dissociation that is produced by these drugs.

4.1. Technical considerations

We used the cat as the animal model because it has well-defined, consolidated sleep and waking states. In addition, this animal model has the advantage that ≈40 Hz oscillations can be clearly observed directly in the raw EEG recordings [16,24]. Finally, the recordings were obtained in a semi-restricted condition, which has the advantage that the differences among states are the states *per se*; postures or movements did not influence the recordings, and movements' artifacts are

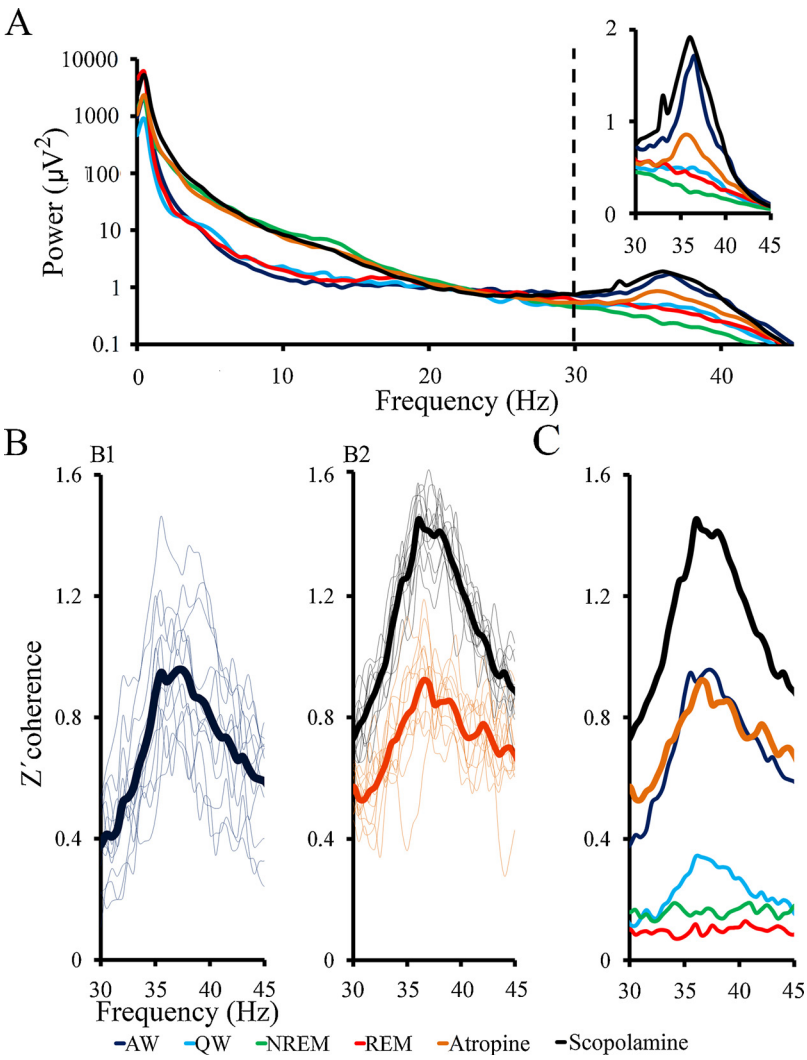

Fig. 6. A. Power spectrum (0–45 Hz) of the posterior-parietal cortex during alert (AW) and quiet (QW) wakefulness, sleep, atropine and scopolamine administration. The inset highlight the low gamma band. B. Twelve profiles of the z´-coherences (thin lines) of a representative pair of recordings (prefrontal and posterior-parietal cortices) as well as the averages of these 12 profiles (thick lines) are shown for AW (B1), and following atropine and scopolamine administration (B2). C. Average gamma z'-coherence profiles' during AW, QW, NREM sleep, REM sleep, atropine and scopolamine administration. Statistical analyses are presented in the Tables. All the analyses are from the same representative animal.

Table 1
Gamma (30–45 Hz) power values during sleep, wakefulness and following atropine administration.

		AW	QW	NREM	REM	A	F
C1	Pp	64.4 ± 2.4*	18.3 ± 4.7*	8.9 ± 0.3*	15.1 ± 0.5*	81.1 ± 3.7	66
	S1	15.3 ± 0.6	13.1 ± 0.9*	7.6 ± 0.3*	13.0 ± 0.6*	17.8 ± 0.8	7
C2	Pf	60.9 ± 3.1*	23.8 ± 3.6	12.5 ± 0.3	20.9 ± 0.4	18.6 ± 1.6	50
	Pp	57.6 ± 2.6*	21.1 ± 3.9	10.3 ± 0.3*	18.5 ± 0.5	23.1 ± 1.3	37
C3	Pp	14.0 ± 0.8*	10.8 ± 0.9	6.5 ± 0.6*	9.7 ± 1.3	9.9 ± 0.8	26
	S1	26.2 ± 1.5*	13.1 ± 0.9*	6.1 ± 0.4	7.7 ± 0.5	8.4 ± 0.8	131
C4	M1	75.8 ± 4.3*	26.7 ± 0.9*	15.8 ± 0.5*	19.6 ± 0.9*	34.3 ± 1.2	73
	V1	57.4 ± 3.0*	8.4 ± 0.6*	5.8 ± 0.91*	10.2 ± 0.5*	28.2 ± 3.6	55
C5	Pf	132.0 ± 4.0*	57.2 ± 5	20.5 ± 1.3*	18.3 ± 1.1*	51.2 ± 7,7	113
	S1	121.2 ± 4.9*	69.8 ± 4.4*	18.6 ± 1.8*	37.0 ± 2.4	38.9 ± 3.9	121
	Pp	174.0 ± 11.2*	79.8 ± 4.4	35.3 ± 3.2*	39.9 ± 2.5*	63.8 ± 5.0	85

The values represent mean ± standard error. The asterisks indicate statistical significance ($p < 0.05$) compared to atropine (A). ANOVA with Tamhane tests. All the analyses have the same degrees of freedom (4 between groups, 55 within groups). Right cortices are shown for C2 to C5, while left cortices are exhibited for C1.

also reduced.

In previous studies, we analyzed the gamma band in the cat up to 100 Hz [24–26]. In these studies, we demonstrated that the narrow 30–45 Hz band is highly modified by alertness or attention. This is not the case for higher gamma band (50–100 Hz), since we only observed that coherence decreased during REM sleep, without significant differences between AW, QW or NREM sleep. Hence, as we did before [27], in the present report we focused in low gamma (30–45 Hz) band. However, we noticed that high gamma coherence under scopolamine/atropine was similar than during AW, QW or NREM sleep (data not shown).

At random sound stimulation produces a large increase in gamma coherence ([24], and Fig. 6B and C). However, in pilot experiments we observed that under atropine/scopolamine the alertness induced by this stimulation did not further increase the gamma coherence (data not shown). Moreover, it is important to comment that in the present report we did not design experiments to explore the sensory evoked/induced potentials in the gamma range, neither in basal nor in drug-induced

Table 2
Gamma (30–45 Hz) power values during sleep, wakefulness and following scopolamine administration.

		AW	QW	NREM	REM	S	F
C1	Pp	64.4 ± 2.4	18.3 ± 4.7*	8.9 ± 0.3*	15.1 ± 0.5*	61.1 ± 5.3	6
	S1	15.3 ± 0.6	13.1 ± 0.9	7.6 ± 0.3*	13.0 ± 0.6	14.8 ± 2.4	64
C2	Pf	60.9 ± 3.1*	23.8 ± 3.6	12.5 ± 0.3*	20.9 ± 0.4	24.8 ± 1.2	71
	Pp	57.6 ± 2.6*	21.1 ± 3.9	10.3 ± 0.3*	18.5 ± 0.5	35.3 ± 1.4	71
C5	Pf	132.0 ± 4.0	57.2 ± 5*	20.5 ± 1.3*	18.3 ± 1.1*	69.1 ± 3.0	187
	S1	121.2 ± 4.9*	69.8 ± 4.4*	18.6 ± 1.8*	37.0 ± 2.4*	38.9 ± 3.9	116
	Pp	174.0 ± 11.2*	79.8 ± 4.4	35.3 ± 3.2*	39.9 ± 2.5*	112.0 ± 5.2	86

The values represent mean ± standard error. The asterisks indicate statistical significance ($p < 0.05$) compared to scopolamine (S). ANOVA with Tamhane tests. All the analyses have the same degrees of freedom (4 between groups, 55 within groups). Right cortices are shown for C2 and C5, while left cortices are exhibited for C1.

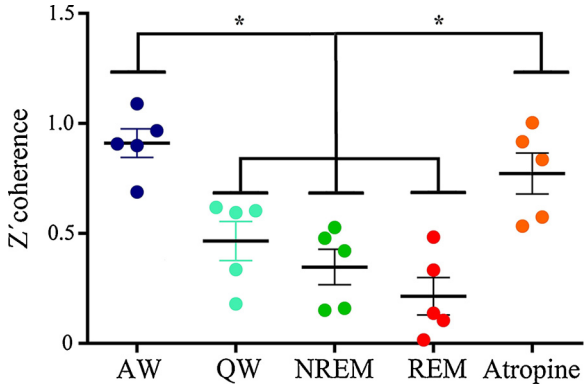

Fig. 7. The plot shows the z'-coherence from intra-hemispheric (Anterior-Posterior), combinations of electrodes during alert (AW) and quiet (QW) wakefulness, NREM and REM sleep, and following the administration of atropine. * $p < 0.0001$, $F_{4,24} = 31.4$, n = 5; rmANOVA and Bonferroni tests.

states.

Acetylcholine produces its biological effects by acting on nicotinic and muscarinic receptors. Five muscarinic receptors (M1–M5) have been cloned [37]. Atropine and scopolamine are non-selective competitive muscarinic antagonists that differ in their pharmacokinetics; the latter has a short half-life and easily permeates the blood-brain barrier, whereas atropine does not [38,39]. Hence, scopolamine has more pronounced and rapid central effects compared to atropine; this property may explain that the differences between both drugs were mainly in quantity, but not in quality.

4.2. Electrocortical and behavior dissociation

As shown in the present and several other studies (see Introduction), behavioral W is present following treatment with muscarinic receptor antagonists. However, subtle cognitive effects have been described in the cat [30]. In fact, scopolamine disrupts the executive phase of predation (i.e., the killing grip) and the consumption of prey without disturbing the preparatory (motivational) phase of predation (usually expressed in interest in the mouse and approaching it by means of crouching, running or jumping) [30].

Classic pharmacological studies have also shown cognitive disturbances in humans treated with atropine [40]. This drug produces a decrease in spontaneous speech and movement, impairment in memory and attention, and drowsiness. However, subjects are able to answer simple questions and perform tasks without the requirement of prolonged attention or memory. They can sit, stand, open or close their eyes or extend their extremities on request, although they move more slowly than in the pre-drug period. Scopolamine also produces sedation, impairment of coordinative and reactive skills, visual disturbances and diminution of short-term memory [41]. Furthermore, the drug affects simple and choice reaction time, number matching and memory scanning tasks. On the contrary, it does not modify word recognition and delayed word recall [39]. Sahakian (1988) suggested that the most prominent effects of scopolamine are those that involve discrimination processes, vigilance, selective attention, as well as consolidation and retrieval of memories [42].

Strikingly, following atropine or scopolamine treatment, the above-mentioned waking behavior in animals and humans is accompanied by sleep spindles and slow waves in the EEG [3,5,6,8,40,43–46]. The present results also demonstrate this fact. However, although we did not perform an in-depth analysis of slow waves and spindles, these waves are similar to those present during NREM sleep.

4.3. Gamma activity nested in slow waves

Figs. 3–5 clearly show that gamma activity is present during NREM sleep. Previous reports have already demonstrated that gamma activity can be observed nested in the slow waves "up states" during anesthesia and NREM sleep [47–51]. In our recordings, gamma oscillations both during atropine/scopolamine and NREM sleep, are associated with the negative component of the slow waves, which probably is associated with the "up states". Hence, it is likely that atropine and scopolamine enhance these oscillations.

Table 3
Gamma (30–45 Hz) z'-coherence values during sleep, wakefulness and following atropine administration.

		AW	QW	NREM	REM	A	F
C1	Ppr-Ppl	1.13 ± 0.03	0.84 ± 0.07	0.77 ± 0.03*	0.55 ± 0.03*	1.52 ± 0.02	11
	S1l-Ppl	0.98 ± 0.04	0.41 ± 0.03*	0.53 ± 0.03*	0.33 ± 0.01*	1.00 ± 0.08	31
C2	Pfr-Ppr	0.90 ± 0.06	0.34 ± 0.05*	0.16 ± 0.01*	0.10 ± 0.01*	0.84 ± 0.03	38
	Ppr-Ppl	0.95 ± 0.04	0.46 ± 0.05*	0.27 ± 0.04*	0.20 ± 0.03*	0.96 ± 0.02	67
C3	Ppr-Ppl	0.43 ± 0.01	0.34 ± 0.04	0.05 ± 0.01*	0.05 ± 0.01*	0.47 ± 0.08	12
	S1r-Ppr	0.97 ± 0.02	0.60 ± 0.04*	0.48 ± 0.01*	0.48 ± 0.01*	0.92 ± 0.08	22
C4	M1r-V1r	0.91 ± 0.02*	0.18 ± 0.01*	0.15 ± 0.01*	0.02 ± 0.01*	0.53 ± 0.05	88
C5	Pfr-Ppr	0.69 ± 0.04*	0.59 ± 0.04*	0.42 ± 0.07	0.14 ± 0.06*	0.57 ± 0.04	215
	S1r-Ppr	0.91 ± 0.04*	0.82 ± 0.06	0.62 ± 0.06*	0.42 ± 0.03*	0.83 ± 0.03	190

The values represent mean ± standard error. The asterisks indicate statistical significance ($p < 0.05$) compared to atropine (A). ANOVA with Tamhane tests. All the analyses have the same degrees of freedom (4 between groups, 55 within groups).

Table 4
Gamma (30–45 Hz) z'-coherence values during sleep, wakefulness and following scopolamine administration.

		AW	QW	NREM	REM	S	F
C1	Ppr-Ppl	1.13 ± 0.03*	0.84 ± 0.07*	0.77 ± 0.03*	0.55 ± 0.03*	1.43 ± 0.02	70
	S1l-Ppl	0.98 ± 0.04*	0.41 ± 0.03*	0.53 ± 0.03*	0.33 ± 0.01*	1.26 ± 0.06	172
C2	Pfr-Ppr	0.90 ± 0.06*	0.34 ± 0.05*	0.16 ± 0.01*	0.10 ± 0.01*	1.30 ± 0.03	179
	Ppr-Ppl	0.95 ± 0.04*	0.46 ± 0.05*	0.27 ± 0.04*	0.20 ± 0.03*	1.09 ± 0.01	200
C5	Pfr-Ppr	0.69 ± 0.04*	0.59 ± 0.04	0.42 ± 0.07*	0.14 ± 0.06*	0.56 ± 0.01	184
	S1r-Ppr	0.91 ± 0.0*4	0.82 ± 0.06	0.62 ± 0.06*	0.42 ± 0.03*	0.92 ± 0.03	178

The values represent mean ± standard error. The asterisks indicate statistical significance ($p < 0.05$) compared to scopolamine (S). ANOVA with Tamhane tests. All the analyses have the same degrees of freedom (4 between groups, 55 within groups).

4.4. Lessons for cognitive functions

The present results strongly suggest that the presence of spindles and delta waves oscillations in the EEG, by their own, are not responsible for the total loss of consciousness that occurs during deep NREM sleep [52]. Nevertheless, although behavioral W is present under atropine/scopolamine, cognition is affected.

Slow waves. It has been hypothesized that the brain's capacity to generate conscious experiences is reduced in the presence of slow waves [23,52,53]. In this regard, thalamo-cortical neurons become hyperpolarized and fire in a burst mode during the slow waves of NREM sleep. This hyperpolarization reduces the transmission of sensory information through the thalamus, which results in the cortex being, at least partially, functionally disconnected from outside sensory experiences [54]. Furthermore, because the level of consciousness depends on the brain's capacity to integrate large amount of information [55], the intracortical reduction of communication during NREM sleep is likely to be directly involved in the loss of consciousness [56].

Gamma activity. In spite of the presence of gamma activity during sleep, recordings with macroelectrodes in the present and previous studies in animals [15,24,57], and humans [58], have shown that during NREM sleep there is a decrease in gamma power compared to W (AW and QW) and REM sleep. In addition, compared to W, long-range gamma coherence or functional connectivity is reduced during NREM sleep [24,57–59]. In contrast, high gamma power and coherence in the EEG are present following muscarinic receptor antagonists' treatment.

Hence, gamma power, which is related to local synchronization at that frequency, and gamma coherent activity, that reflects functional interactions between "distant" cortical areas, may contribute in a significant way to the maintenance of a functional cognitive state during W; i.e. it is likely critical for consciousness. In fact, studies utilizing general anesthetics as an "off-switch" for consciousness, have shown that a strong reduction in gamma coherence (mainly between far cortical regions) is correlated with the loss of awareness [60–62]. Therefore, it is possible that in order to lose consciousness, as in deep NREM sleep, both slow waves and a decrease in gamma coherence are required.

Based on experiment in rats, Vanderwolf (2000) showed that while during W there is a continuous cortical gamma activity, during anesthesia as well as under scopolamine, predominates an interrupted pattern of gamma waves [51]. The author suggested that this last profile may not support a normal cognitive function. A detailed analysis of the temporal patterns of the gamma "bursts" during W, sleep, and under different drugs that affect cognition is still lacking.

4.5. The activation of muscarinic receptors is not needed for gamma activity

Gamma-band rhythmogenesis is inextricably tied to perisomatic inhibition in the cerebral cortex, wherein the key ingredient is $GABA_A$-receptor mediated inhibition [63]. GABAergic cortical neurons, glutamatergic cortico-cortical neurons as well as glutamatergic thalamo-cortical neurons have been suggested as the anatomical substrate for gamma band oscillations and coherence in the EEG [20,63]. Intrinsic neuronal properties of cortical neurons that determine that when activated by subthreshold current injection produce membrane potential oscillations at or near 40 Hz may be also involved [64]. In addition, thalamo-cortical activity is modulated by regulatory systems that consist of small groups of neurons that, with a common neurotransmitter, project to many regions of the central nervous system [1,2]. Cholinergic, monoaminergic and hypocretinergic neurons are part of these systems. By acting through the thalamus and/or cortex, these systems promote W and may regulate the appearance of gamma oscillations and coherence (and therefore cognitive functions). In fact, activation of the mesencephalic reticular formation, where part of the activating systems are located, was shown to facilitate oscillatory activity in the gamma frequency range and to enhance the stimulus-specific synchronization of neuronal spike responses in the visual cortex of cats [65].

Cholinergic neurons of the laterodorsal and pedunculo-pontine tegmental nucleus (LDT and PPT) project to the non-specific thalamo-cortical system where they promote cortical activation [1,2]. Cholinergic neurons discharge at high rates during W and REM sleep [66]. In addition, Garcia-Rill and co-workers sustain that neurons within the PPT generate beta/gamma band activity during waking [67,68]. These neurons have low-gamma membrane oscillations that are mediated by voltage-dependent high-threshold N- and P/Q-type calcium channels modulated by G proteins. In anesthetized animals, cholinergic projection neurons in the PPT fire rhythmically during cortical slow oscillations, and predominantly discharge in time with the phase of the slow oscillations supporting nested gamma oscillations (30–60 Hz) [49]. Also, cholinergic neurons of the basal forebrain have long been known to have an important role in cortical activation [69]. However, we found that atropine and scopolamine are unable to prevent coherent gamma oscillation. Then, this result suggests that the acetylcholine, acting through muscarinic receptors, is not critical to generate coherent gamma activity. In agreement with these findings, the role of cholinergic basal forebrain neurons in the generation of gamma oscillations in the EEG has been negated [70]; however, basal forebrain GABAergic neurons that project to the cortex play an important role in the generation of gamma activity [70]. The fact that cholinergic neurons are active during REM sleep [66], and gamma coherence in virtually absent during this behavioral state [24,26,27,57,58,71,72], is in total agreement with the present results. However, following antimuscarinic treatment, ensembles of cholinergic (through nicotinic receptors), glutamatergic and GABAergic neurons of the PPT may still transfer gamma actitity to the cortex through the intalaminar thalamic nuclei, or by the regulation of non-cholinergic neurons of the basal forebrain [73,74]. Finally, also in accordance with our results, systemic atropine administration in exploring mice increases the amplitude of gamma oscillations of the CA1 region of the dorsal hippocampus [75].

Monoaminergic and hypocretinergic systems, that may remain active following the administration of muscarinic receptor antagonists, might induce gamma power and gamma coherence acting through the thalamus and/or cortex despite the presence of sleep spindles and slow waves. New sets of experiments are needed to test this hypothesis.

4.6. Pathology, toxicology and drug abuse

A decrease in cortical cholinergic activity is present in Alzheimer disease (AD); this illnesses involve a failure to focus on the most relevant information, and difficulty in maintaining an appropriate stream of awareness [76]. Interestingly, AD patients increased sensory-evoked and event-related gamma coherence values compared to healthy controls [77].

Anticholinergic drugs have been used for recreational or ritualistic purposes. One of the most widely described religious or magical experiences dating back to ancient times is the alteration of consciousness with the induction of hallucinations by a member of the Solanaceae family of plants (Belladonna, Henbane or Datura), which contain scopolamine, atropine and other closely related alkaloids [76]. Similar effects occur with the intoxication either with prescribed anti-muscarinic medical drugs [76]. Perhaps the most extraordinary example of cognitive dysfunction is the criminal use of anticholinergic substances that are present in Datura extracts (called "burundanga"), to induce amnesia and submissive behavior or "obedience" in victims [78,79]. Our results that demonstrate the presence of coherent gamma activity following anti-muscarinic receptor treatment; this result is important to advance our understanding of the syndromes that occur when the central cholinergic system is dysfunctional.

Finally, psychosis is associated with disturbances in gamma coherence [80–82]. Interestingly, second generation antipsychotic drugs such as clozapine and olanzapine have an important antagonism on M1 receptors [83]. Hence, due to antimuscarinic antagonists conserve gamma coherence (present results), it would be interesting to know if the benefits of clozapine and olanzapine is in part associated with their impact on gamma activity.

5. Conclusions

Although atropine and scopolamine produced an EEG with slow waves and sleep spindles that resemble NREM sleep, functional interactions between cortical areas in the gamma band remained high, similar to AW. This phenomenon could explain the dissociation between the EEG and behavior that is elicited by muscarinic receptor antagonist drugs.

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