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Event-related oscillations (ERO) during an active discrimination task: Effects of lesions of the Nucleus Basalis Magnocellularis

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Abstract

The cholinergic system in the brain is involved in attentional processes that are engaged for the identification and selection of relevant information in the environment and the formation of new stimulus associations. In the present study we determined the effects of cholinergic lesions of nucleus basalis magnocellularis (NBM) on amplitude and phase characteristics of event related oscillations (EROs) generated in an auditory active discrimination task in rats. Rats were trained to press a lever to begin a series of 1K Hz tones and to release the lever upon hearing a 2 kHz tone. A time-frequency based representation was used to determine ERO energy and phase synchronization (phase lock index, PLI) across trials, recorded within frontal cortical structures. Lesions in NBM produced by an infusion of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) resulted in (1) a reduction of the number of correct behavioral responses in the active discrimination task, (2) an increase in ERO energy in the delta frequency bands (3) an increase in theta, alpha and beta ERO energy in the N1, P3a and P3b regions of interest (ROI), and (4) an increase in PLI in the theta frequency band in the N1 ROIs. These studies suggest that the NBM cholinergic system is involved in maintaining the synchronization/phase resetting of oscillations in different frequencies in response to the presentation of the target stimuli in an active discrimination task.

Keywords

Event-related potentials; Electroencephalogram; cholinergic system; event related oscillations; phase lock index

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Contributors

All authors have given intellectual contributions to the study and the paper and have approved the final manuscript. Cindy L. Ehlers was responsible for the study design and preparation of the manuscript. Manuel Sanchez-Alavez was responsible for data analyses and preparation of the manuscript.

Conflict of Interest

Dr. Ehlers work has been funded by the NIH. She has received compensation as a consultant from Neurocrine Biosciences and Raptor Pharmaceutical Corp. in capacities not related to the subject of the report. Dr. Manuel Sanchez-Alavez, declare no potential conflicts of interest.

1. Introduction

Central cholinergic pathways, in the cortical and subcortical regions, (Woolf, 1991, 1996) contribute to a number of cognition abilities such as learning, memory, and attention (Furey, 2011; Klinkenberg et al., 2011; Ortega et al, 1996; Pepeu and Giovannini, 2004; Sarter et al., 2003) . The nucleus basalis magnocellularis (NBM) is a major source of cholinergic innervation to the cerebral cortex and has been shown to play a key role in modulating attentional processes (Harati et al., 2008; Lehman et al., 2003; McGaughy et al., 2002; Risbrough et al., 2002; Turchi and Sarter, 1997). It has been shown that cortical acetylcholine (ACh) efflux increases during early stages of learning, when attentional processes are engaged for the identification and selection of relevant information in the environment, and during the formation of new stimulus associations (Acquas et al., 1996; Himmelheber et al., 2000; Inglis and Fibiger, 1995; Orsetti et al., 1996). It has also been shown that performance, in a task incorporating components of selective and sustained attention, is disrupted by excitotoxic lesions of the basal forebrain (McGaughy and Sarter, 1998; McGaughy et al., 1996; Muir et al., 1994, 1995). Taken together these data support the hypotheses that cortical cholinergic transmission is important for attentional functions (Muir et al., 1992; Sarter and Bruno, 1997).

Ljubojevic and colleagues (2014) demonstrated that cholinergic neurotransmission in the fronto-parietal cortical network contributes to the attentional processing of stimuli in a number of different modalities (i.e. in a supramodal manner). Additionally, it has been demonstrated that bilateral lesions of the NBM affect both visual and olfactory attention (Ljubojevic et al., 2014) when rats are evaluated in a sustained attention task that involves a briefly appearing target stimulus (Robbins, 2002). These findings are also in accordance with human studies showing an activation of frontoparietal cortical attentional network during sustained attentional processing of stimuli of different modalities (Cohen et al., 1992; Fink et al., 1997; Lückmann et al., 2014).

Recently, spontaneous electroencephalography (EEG), sensory-evoked oscillations, and event-related oscillations (EROs) have emerged as potential functional tools capable of indexing hypofunction of the cholinergic system in animal models (Sanchez Alavez et al., 2014). The search for the functions of brain oscillations is now an important tool in neuroscience, since oscillatory activity in various frequency bands may reflect different aspects of information processing (Ba ar-Eroglu et al., 1992; Karakas and Basar, 2006) and memory formation (Klimesch et al., 1997). The stimuli that evoke ERPs components influence oscillatory changes within the dynamics of ongoing EEG rhythms (Basar-Eroglu et al., 1991; Demiralp and Ademoglu, 2001; Ehlers et al., 2012; Karakas et al., 2000a, 2000b; Schurmann and Basar, 2001; Yordanova et al., 2002), and this synchronization or enhancement of ongoing EEG oscillations by a time locked cognitive and/or sensory process is termed event-related oscillations (Basar et al., 2000; Begleiter and Porjesz, 2006; Roach and Mathalon, 2008). EROs are typically estimated by a decomposition of the EEG signal into phase and magnitude information for a range of frequencies and then changes in those frequencies are characterized with respect to their energy (amplitude) and phase relationships over a millisecond time scale with respect to task events (Ehlers et al., 1994). EROs have been demonstrated to be sensitive measures of both normal (Basar et al., 1999;

Gevins, 1998) and abnormal cognitive functioning (Begleiter and Porjesz, 2006; Criado and Ehlers, 2009, 2010a; Ehlers et al., 2012; Porjesz and Begleiter, 2003). Additionally, they may serve as biomarkers for neuropsychiatric diseases in disorders such as: attention deficit hyperactivity disorder, Alzheimer's disease, bipolar disorder, schizophrenia (for review see (Basar et al., 2013; Yener and Basar, 2013), and alcoholism (Andrew and Fein, 2010; Criado and Ehlers, 2009, 2010a, 2010b; Ehlers et al., 2010, 2012; Kovacevic et al., 2012; Rangaswamy and Porjesz, 2008).

Until recently, active discrimination tasks have not been utilized to generate EROs in animal models. We have previously demonstrated that late positive components of event-related potentials can be generated using active discrimination tasks in rodents (see Ehlers and Chaplin, 1992; Ehlers et al., 1994, 1998). The aims of the present study were to extend those studies to 1) describe the amplitude and phase characteristics of ERO oscillatory activity in the delta, theta, alpha, beta and gamma frequency bands in the rat frontal cortex, elicited by an active acoustic discrimination task. Secondly, we examined whether selective cholinergic lesions of the nucleus basalis magnocellularis (NBM) altered ERO responses to the task.

2. Method

2.1 Animal subjects

The present set of analyses was accomplished from the same datasets that were used to generate the cortical ERP data reported in a previous publication (Robledo et al., 1998). In brief, eighteen male Wistar rats (Charles River, USA) weighing 250–300 g were utilized in the study. The work described herein adheres to the guidelines stipulated in the NIH *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 80-23, revised 1996) and was reviewed and approved by The Scripps Research Institute's Institutional Animal Care and Use Committee.

Rats were housed in pairs on a 12 h light-dark cycle (light on at 6 am) and food restricted to 85% of their free-feeding weight throughout the experiment. Rats were first trained to obtain food reinforcement in standard operant boxes (Coulbourn Instruments, Whitehall, PA). In the first session they were placed in the boxes for ten minutes with 8 pellets of food in the reinforcement tray. In the next session, rats received 100 pellets of food (45 mg each; Noyes) each delivered every 45 s. Subsequently, rats were trained to press a lever to obtain food on a fixed ratio 1 schedule of reinforcement (FR1) in five 30 minute sessions. Only those rats that learned to press the lever for food reinforcement were implanted with bilateral cannulae above the NBM and recording electrodes in the frontal cortex.

2.2 Implantation of electrodes and cannulae

Rats were deeply anesthetized with Nembutal (50mg/kg, intraperitoneally) and surgically implanted with bilateral cannula above the nucleus basalis magnocellularis (NBM: AP -0.8 ; ML ± 2.5 ; DV -4.3 mm, Paxinos and Watson, 1986) together with stainless steel single-wire recording electrodes placed in the skull overlying the frontal (AP: 3.0 mm, ML: ± 3.0 mm, FR1) cortex. A midline screw “reference” electrode was placed 3 mm posterior to lambda in the skull overlying the cerebellum. For the recordings, electrode connections were made to a

multipin amphenol connector and the assembly was anchored to the skull with dental acrylic and anchor screws. Rats were allowed to recover from surgery for eleven days.

2.3 Behavioral training

Following recovery from surgery, rats were trained on an auditory discrimination task. The task used was a two tone discrimination task, based on a reaction time paradigm described by Almaric and Koob (1987) and modified from Conde et al. (1978). Training was carried out in an operant chamber equipped with food pellet dispenser, a retractable lever, a reinforcement tray, and a speaker located in the panel directly above the tray for presentation of the auditory stimuli. This task required the rat to press a lever to initiate the presentation of a non-target stimulus and to release the lever within 1 sec following the presentation of a target stimulus. The time elapsed between the two stimuli was varied randomly from 250 to 1000 ms. If the rats successfully released the lever within 1 sec following presentation of the target stimulus they were reinforced with a food pellet (Noyes 45mg). However if rats failed and released the lever before the onset of the target tone, or more than 1 sec after the presentation of the target tone, no reward was given. One hundred trials were presented per session. When a rat had reached a 60% success rate on this procedure for three consecutive days EROs were recorded for baseline. Then rats were divided into two groups: sham-operated controls (n=8), and NBM-lesion groups (n=10). EROs were recorded 15 days post-lesion.

2.4 ERO recording procedure

Rats were retrained on the auditory discrimination task for 6 consecutive days prior to the recordings. For ERO recordings rats were placed singly in the operant chambers and a connector (Amphenol) attached to a microdot cable was used to transfer the EEG signals. The bandpass for recordings was set at 0.53 – 35 Hz. A Macintosh computer controlled the presentation of stimuli. EROs were elicited by auditory stimuli that were presented through a small speaker centered approximately 20 cm above the rat's head.

One hundred trials were presented consisting each of two tones, the non-target tone (20 ms, 1 kHz, 60 dB SPL) which was not rewarded and, the target tone (20 ms, 2 kHz, 60 dB SPL) which was rewarded with a food pellet. Individual trials were 1000 ms in duration (200 ms pre-stimulus + 800 ms post-stimulus) and were separated by variable intervals ranging from 500 to 1000 ms. Non-target tone + non-target tone were interspersed with non-target + target tone such that no two non-target + target tone occurred successively. The EEG amplifier input range corresponding to the full range of the 12-bit analog-to-digital converter was about ± 250 microvolts (μV). Periodic calibration results were used to scale the digitized EEG to microvolts. An artifact rejection program was utilized to eliminate individual trials in which the EEG exceeded $\pm 400 \mu\text{V}$. Potential artifacts identified by computer software were excluded only after visual analysis of raw EEG. Trials containing excessive movement artifact were eliminated prior to averaging (<5% of the trials). ERPs trials were digitized at a rate of 256 Hz.

2.5 ERO energy and PLI analyses

Data from single trials generated by the stimuli were entered into the time frequency analyses algorithm. The $\mathcal{S}$ -transform (ST), a generalization of the Gabor transform (Gabor, 1946) was used (see Stockwell et al., 1996).

$$S\left[jT, \frac{n}{NT}\right] = \sum_{m=0}^{N-1} H\left[\frac{m+n}{NT}\right] e^{\frac{-2\pi^2 m^2}{n^2}} e^{\frac{i2\pi m j}{N}} \quad n \neq 0$$

The $\mathcal{S}$ transform mathematically resembles the continuous wavelet transform but it uses Gaussian windows which do not meet a requirement of wavelet analysis, and it includes a “phase correction” that is not part of wavelet analysis. The actual use of the $\mathcal{S}$ -transform was simplified by performing first a forward Fourier transform of the time series. Then, for each frequency of the Fourier transform, summing the results of multiplication by a set of Fourier transforms of Gaussian windows of varying width. Finally, for each of these sums, taking the inverse Fourier transform. The equation for calculation of the $\mathcal{S}$ -transform of discrete time series $h(kT)$ at time jT and frequency n/NT is where T is the sample period of the discrete time series, j is the sample index, N is the number of samples in the time series, n is the frequency index, and $H[\]$ is the Fourier spectrum of the discrete time series. The $\mathcal{S}$ -transform results in a time-frequency representation of the data. The exact code we used is a C language, $\mathcal{S}$ -transform subroutine available from the NIMH MEG Core Facility web site (<http://kurage.nimh.nih.gov/meglab/>). This code is specifically for use with real time series, so it sets the input imaginary values, required by the $\mathcal{S}$ -transform, to zero, and it always uses the Hilbert transform so that each of the complex output time series is an analytic signal.

To reduce anomalies in the $\mathcal{S}$ -transform output at the beginning and the end of the output time series, we used a Hanning window over the initial and final 100 msec of the input time series. The output of the transform for each stimulus and electrode site was calculated by averaging the individual trials containing the time-frequency energy distributions. To quantify $\mathcal{S}$ -transform magnitudes, a region of interest (ROI) was identified by specifying the band of frequencies and the time interval contained in the rectangular ROI. The time-frequency points saved from each $\mathcal{S}$ transformation are from 100 ms before to 900 ms after the onset of the stimulus, and from 1 Hz through 50 Hz at intervals of 0.5 Hz. Energy is the square of the magnitude of the $\mathcal{S}$ -transform output in a time frequency region of interest. The $\mathcal{S}$ -transform output for a time/frequency ROI, for a specific EEG lead, is proportional to the input voltage of the lead over the time/frequency interval. The $\mathcal{S}$ -transform magnitude squared for a time/frequency interval is therefore proportional to volts squared. These analyses are similar to what has been previously described (Jones, 2004).

An $\mathcal{S}$ transformation at time t and frequency f has real and imaginary parts:

$$S(t, f) = \text{Re}S(t, f) + i\text{Im}S(t, f)$$

where i is the square root of minus 1. The cosine and sine of the phase angle at this time-frequency point are:

$$\begin{aligned}\cos \phi(t, f) &= \operatorname{Re} S(t, f) / |S(t, f)| \\ \sin \phi(t, f) &= \operatorname{Im} S(t, f) / |S(t, f)|\end{aligned}$$

where the vertical bar pair indicates magnitude, here and below.

ERP trials are averaged by summing separately the real and imaginary parts of the S transform outputs, and dividing each by the sum over trials of the magnitudes of S transform outputs. The sums over trials of the real and imaginary parts of the S transform outputs are the sides of a right triangle and the sum of magnitudes is the hypotenuse. From this, the angles of the triangle of the sums are calculated:

$$\begin{aligned}\langle \cos \phi(t, f) \rangle &= \sum \operatorname{Re} S_n(t, f) / \sum |S_n(t, f)| \\ \langle \sin \phi(t, f) \rangle &= \sum \operatorname{Im} S_n(t, f) / \sum |S_n(t, f)|\end{aligned}$$

where an angle bracket pair indicates mean value from S transforms, and a vertical bar pair indicates magnitude, here and below.

PLI is a measure of synchrony of phase angle over trials, as a function of frequency and of time relative to the start of the stimulus for each trial. The range of PLI is from zero to 1.0, with high values at a time and frequency indicating little variation, among trials, of phase angle at that time and frequency. PLI is defined as:

$$PLI(t, f) = |\langle \cos \phi(t, f) + i \sin \phi(t, f) \rangle|$$

where the angle bracket pair indicates mean value over eligible trials, here and below. Eligibility depends on the stimulus type and the absence of significant artifact. This definition is mathematically equivalent to the definition in Schack and Klimesch (2002).

Rectangular regions of interest (ROIs) were defined within the time-frequency analysis plane by specifying, for each ROI, a band of frequencies and a time interval relative to the stimulus onset time. We reported previously that NBM lesion modifies N1 and P3b component amplitudes of the ERP (Robledo et al., 1998). We used the time points for N1 (50-200 ms), P3a (275-325 ms) and P3b (350-400 ms) that were determined from the grand averages of the event related potentials in the frontal cortex to construct the ROIs in the different frequency bands. Time 0 in these definitions is the onset of the target stimulus. The 13 ROIs were: ROI1 (delta band, 1-4 Hz, 200-500 ms), ROI2 (theta band N1 segment, 4-7 Hz, 50-200 ms), ROI3 (theta band P3a segment, 4-7 Hz, 275-325 ms), ROI4 (theta band P3b segment, 4-7 Hz, 350-400 ms), ROI5 (alpha band N1 segment, 7-13 Hz, 50-200 ms), ROI6 (alpha band P3a segment, 7-13 Hz, 275-325 ms), ROI7 (alpha band P3b segment, 7-13 Hz, 350-400 ms), ROI8 (beta band N1 segment, 13-30 Hz, 50-200 ms), ROI9 (beta band P3a segment, 13-30 Hz, 275-325 ms), ROI10 (beta band P3b segment, 13-30 Hz, 350-400 ms), ROI11 (gamma band N1 segment, 13-30 Hz, 50-200 ms), ROI12 (gamma band P3a segment, 13-30 Hz, 275-325 ms), and ROI13 (gamma band P3b segment, 13-30 Hz, 350-400 ms). These regions were chosen apriori to coincide with the major EEG frequencies and the

latency windows of the N1, P3a and P3b components in the rat reported previously in Robledo et al. (1998). Using mean values over trials, the maximum values were calculated for each ROI, for each electrode location for energy and phase locking index amplitude.

2.6 Lesion and perfusion

The methods for performing the lesions and perfusions have been previously reported (Robledo et al., 1998). Briefly, under Nembutal anesthesia (1 ml/kg) rats were infused through previously implanted guide cannula with 0.5 μ l per side 0.01 M AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; Cambridge Research Biochemicals, UK) (Muir et al., 1994) into the NBM (3 mm below cannula at DV = -7.3), and the controls were infused with vehicle (0.2 M PB). ERO trials were conducted 15 days following the sham/lesion surgery. At the end of the study rats were euthanized and their brains were extracted rapidly and dissected on wet ice. The frontal and parietal cortices and hippocampus were removed and choline Acetyltransferase (ChAT) activity was measured. In brief, extracted tissue was sonicated in 400 μ l of 50nM phosphate buffer (pH 7.4). ChAT activity was measured by the incorporation of 14 C-acetyl coenzyme A into 14 C-Ach (Robledo et al., 1998). The assay is based on the transfer of the radiolabeled acetyl moiety from acetyl CoA to choline, and separation of radiolabeled 14 C-acetyl CoA from the radiolabeled product, 14 C-Ach (Fonnum, 1969).

2.7 Statistical analyses

Statistical analyses were based on the specific aims. For the first aim, the target and non-target tones were analyzed at baseline (pre-lesion), in order to determine the effects of tone discrimination and performance on EROs on N1, P3a and P3b segments. A Multivariate Analysis of Variance (MANOVA) was used to test for differences between the target and non-target tones for energy and PLI in the 13 regions of interest (ROIs). For the second aim the effects of NBM lesions vs. baseline on EROs were evaluated. Estimated marginal mean values from 2 (group) $\times$ 2 (tone) ANOVA for the event-related oscillations energy equivalents in frequency bands (δ , θ , α , β , and γ) of sham operated (n=8) and NBM lesioned (N=10) subjects at 15 days post lesion. Tone (non target, target) and group (sham, lesion) were compared at 200-500 ms (delta frequency band only) and at N1 (50-200 ms range), P3a (275-325 ms range) and P3b (350-400 ms range) in the theta, alpha, beta and gamma bands. Lesion effects were determined using a two way ANOVA (group $\times$ tone), group (sham vs. NBM-lesion), tone (non target, target). One-way ANOVA was used to assess lesion site differences in ChAT. For these analyses, post-hoc P-value was set at $P < 0.01$ to determine the level of statistical significance and control for multiple comparisons. Values are mean $\pm$ standard error of the mean (SEM).

3. Results

3.1 NBM-lesion decreases ChAT activity in frontal and parietal cortex

Choline Acetyltransferase (ChAT) activity (nmoles Ach/h/mg prot) was measured in the frontal cortex and central cortex of sham and NBM lesion rats (Robledo et al., 1998). Results of those determinations were analyzed using two-way Analysis of Variance (ANOVA) that revealed a significant main effect of group (sham, NBM-lesion) ($F = 156.2$,

$P < 0.0001$), region (frontal cortex, central cortex) ($F = 54.57$, $p < 0.0001$), and a Group $\times$ Region interaction ($F = 0.0003$, $P = 0.98$). Compared to the sham group, the NBM-lesion was found to significantly reduce frontal cortical ChAT activity (34.6 ± 3.5 %; $P < 0.0001$, sham 59.31 ± 1.3 vs NBM lesion 38.8 ± 2.1 nmoles Ach/h/mg prot) and central cortical ChAT activity (44.8 ± 4.0 %; $P < 0.0001$, sham 47.2 ± 1.1 vs NBM lesion 26.6 ± 1.6 nmoles Ach/h/mg prot).

3.2. NBM-lesion induced changes in correct behavioral responses to the target tone

The task utilized required rats to press a lever to initiate the presentation of a non-target stimulus and to release the lever within 1 sec following the presentation of a target stimulus. Performance in this task was evaluated using a one-way ANOVA. A significant effect of NBM lesion in reducing the number of correct responses to the target tone was observed ($*p < 0.01$, Figure 1A). The reaction time in ms that measures the release on the lever upon hearing the target tone was not significant (Figure 1B) in NBM-lesion group compared to sham operated group.

3.3 ERO energy and Phase locking to the target and non target tone

Multivariate Analysis of Variance (MANOVA) with ERO energy as dependent variable revealed a significant main effect of tone (Wilks' Lambda $F = 2.85$, $p < 0.015$) in the frontal cortex, as seen in Figure 2. Post hoc pairwise comparisons showed higher ERO energy in the frontal beta in the N1 ($F = 6.77$, $*P = 0.014$) P3a ($F = 9.51$, $**P = 0.004$) and P3b ($F = 9.89$, $**P = 0.003$) intervals and in frontal gamma in the N1 ($F = 7.57$, $**P = 0.009$) P3a ($F = 12.22$, $**P = 0.001$) and P3b ($F = 20.23$, $**P < 0.001$) intervals that occurred in response to the target tone (TT) compared to the non target tones (NTT). These results are presented in Figure 2A.

MANOVA with phase locking index (PLI) as dependent variable revealed a significant main effect of tone in the frontal cortex (Wilks' Lambda $F = 5.144$, $p < 0.0001$). Post hoc pairwise comparisons showed higher ERO PLI that occurred in response to the target tone (TT) compared to the non target tones (NTT) in N1, P3a and P3b intervals in the following ROI time frequency intervals: theta band N1 ($F = 10.23$, $**P = 0.003$), alpha band N1 ($F = 10.38$, $**P = 0.003$), alpha band P3a ($F = 7.79$, $**P = 0.009$), alpha band P3b ($F = 8.01$, $**P = 0.008$), beta band N1 ($F = 9.25$, $**P = 0.005$), beta band P3b ($F = 22.04$, $**P < 0.001$), gamma band N1 ($F = 10.86$, $**P = 0.002$), gamma band P3a ($F = 8.45$, $**P = 0.006$), gamma band P3b ($F = 13.80$, $**P = 0.001$). These results are presented in Figure 2B.

3.4. NBM-lesion induced changes in ERO energy in response to the target tone

Figure 3 shows the grand averages of the color equivalent of energy values for the sham operated and NBM-lesion rats at baseline and 15 days following the lesion. We estimated the Marginal Means by UNIANOVA to determine whether there were significant differences in energy and PLI between sham and NBM lesioned animals in each frequency band (δ , θ , α , β , and γ) at 15D (15 days post lesion), these data are presented in figure 4A. A significant increase was seen in NBM lesioned rats as compared to shams at 15 days post lesion: delta ERO energy ($F = 9.6$, $*P = 0.004$), theta ERO energy in the N1 region ($F = 8.5$, $*P = 0.006$), theta ERO energy in the P3a region ($F = 7.8$, $*P = 0.009$), theta ERO energy in the P3b region ($F = 7.4$, $*P = 0.01$), alpha ERO energy in the N1 region ($F = 10.59$, $*P = 0.003$), alpha ERO

energy in the P3a region ($F=10.34$, $*P=0.003$), alpha ERO energy in the P3b region ($F=10.79$, $*P=0.002$), beta ERO energy in the N1 region ($F=13.43$, $*P=0.001$), beta ERO energy in the P3a region ($F=13.13$, $*P=0.001$), and beta ERO energy in the P3b region ($F=13.66$, $*P=0.001$). A significant effect of tone on energy was also seen 15 days post lesion in gamma in the P3a region ($F=7.9$, $P<0.01$) and gamma in the P3b region ($F=18.6$, $P<0.0001$).

Figure 4B also shows the results of analyses of phase locking index values in the sham and lesioned animals at 15 days following the lesion. UNIANOVA revealed a significant increase in theta ERO phase locking index in gamma in the N1 region ($F=8.54$, $*P=0.006$). A significant effect of tone on PLI was also seen 15 days post lesion in delta ERO energy ($F=16.4$, $**P=0.00001$), theta ERO energy in the N1 region ($F=11.3$, $*P=0.002$), theta ERO energy in the P3a region ($F=13.4$, $*P=0.001$), theta ERO energy in the P3b region ($F=9.34$, $*P=0.005$), alpha ERO energy in the N1 region ($F=20.5$, $*P=0.00001$), alpha ERO energy in the P3a region ($F=9.07$, $*P=0.005$), alpha ERO energy in the P3b region ($F=12.8$, $*P=0.001$), beta ERO energy in the N1 region ($F=16.8$, $*P=0.0001$), beta ERO energy in the P3a region ($F=12.34$, $*P=0.001$), beta ERO energy in the P3b region ($F=11.12$, $*P=0.002$), gamma in the N1 region (32.7 , $*P<0.0001$), gamma in the P3a region ($F=21.6$, $P<0.001$) and gamma in the P3b region ($F=28.2$, $P<0.0001$).

4. Discussion

The purpose of the present study was to investigate oscillatory activity in the delta, theta, alpha, beta and gamma frequency bands in the frontal cortex of adult rats, before and after selective cholinergic lesions of the nucleus basalis magnocellularis, during the performance of an active discrimination task (Robledo et al., 1998).

Network oscillations provide a mechanism to functionally link ensembles of neurons from discrete and regulatory pathways into complex interplay during information processing and represent neurophysiological correlates of human information processing and cognitive function (Basar et al., 1999, 2001a; Karakas et al., 2000b). ERO energy and phase locking of frequency specific, neuro-oscillatory activity within and between neural assemblies may underlie the processes whereby the brain organizes and communicates information (Barutcu et al., 2013; Basar et al., 1999; Roach and Mathalon, 2008; Sauseng et al., 2007), and represents a methodology whereby neuronal synchrony and/or phase resetting can be quantified and compared among experimental conditions in both human and animals providing a translatable measure to explore the neural basis of behavior (Basar and Guntekin, 2008; Sazonov et al., 2009; Thatcher, 2012).

By using the active auditory task we found that the target stimulus produced a robust and highly significant increase in both energy and phase locking index of EROs. The most likely explanation of this finding is that it represents a change in neural state associated with attending to an environmentally relevant stimulus, since in our active discrimination task requires active response to the target stimuli in order to release the lever for food reward. Following the NBM lesion differences in ERO energy were not seen in response to the target tone, however, large robust differences in PLI remained despite the lesion. This suggests that

PLI may be a better index of cognitive responses than energy. Higher phase-locking to target vs. non target tones in a number of ERO frequency ranges has been reported previously in human subjects (Ehlers et al., 2012; Yordanova et al., 1997; Yordanova et al., 2002; Muller and Anokhin 2012). In most cases the authors ascribed the differences in phase locking to the response requirements, and or salience of the stimulus. We have also seen significant changes in energy and phase locking in passive tasks in rodents (Ehlers et al., 2014), although not as robustly as what is seen in the present study using a task with an active response requirement.

4.1 NBM-lesion induced changes in ERO energy in the delta frequency band

After lesioning the NBM, ChAT activity decreased in average 34.6 % in the frontal cortex whereas it decreased 44.8 % in the central cortex (Robledo et al., 1998). Correct response to the target tone decreased only ~10% without affecting the timing to execute the task in response to the target tone. Time-frequency analysis shows that ERO energy in delta frequency band increased significantly following lesions to the NBM. It has been suggested that delta ERO responses are mainly involved in signal matching, decision making and surprise (Ba ar-Eroglu et al., 1992). According to Knyazev (2012), functional delta oscillations appear to be implicated in the synchronization of brain activity in motivational processes associated with reward and in cognitive processes related to attention and the detection of motivationally salient stimuli in the environment (Knyazev, 2007; Knyazev et al., 2009). Delta oscillations have also been shown to correlate with the inhibition of non-relevant stimuli (Basar et al., 2001c; Harmony et al., 1996). Our studies in animal models support these hypothesis and further suggest that lesioning of the NBM could extend the inhibition of responses to include relevant stimuli. Moreover, we have shown, in a previous study, that lesioning the NBM increases ERO energy in the delta frequency band in rats that are subjected to a passive acoustic oddball paradigm (Sanchez-Alavez et al., 2014). Therefore we suggest that lesioning of the NBM may increase delta ERO energy independent of task. We have also found that this increase in delta energy may be specific to NBM structure as lesions of the medial septum do not result in changes in delta ERO energy (Sanchez-Alavez et al., 2014).

4.2 NBM-lesion induced changes in ERO energy in N1, P3a and P3b components

Our previous studies have demonstrated that NBM lesions can change the morphology of the N1 and P3b components of the ERP (Robledo et al., 1998). In the present study we found that there was a significant change in theta oscillations in N1, P3a and P3b regions of interest. Oscillations in the theta frequency ranges have been associated with signal detection, decision-making, conscious awareness, recognition memory and episodic retrieval (Basar et al., 2001a, 2013; Gevins, 1998; Klimesch et al., 2005). ERO studies in humans have shown decreased responses in fronto-central regions of the brain in delta and theta frequencies some neuropsychiatric disorders (Yener and Basar, 2013). In our study NBM lesions induced increases in theta ERO energy and in phase locking index in the N1 region. NBM lesions also produced increases in P3a and P3b region theta ERO energy but did not produce changes in PLI in that frequency range. Our findings, of increased theta PLI following the target tone independent of the lesion are consistent with a previous study that evaluated phase locking of EROs using a complex motor-learning task (Sauseng et al., 2007)

where long-range theta phase coherence was stronger in the novel condition compared to learned sequences, independent of task-difficulty.

Event-related alpha oscillations have been attributed to attentional resources, semantic memory, and stimulus processing (Basar et al., 1997; Hanslmayr et al., 2005; Klimesch et al., 2004). Alpha synchronization also seems to be sensitive to cholinergic treatment in Alzheimers disease (Yener and Basar, 2013). Beta and gamma oscillations have been associated with sensory integrative processes (Basar, 2013; Basar et al., 2001b; Schürmann and Basar, 2001). Some studies have demonstrated that cholinergic neuromodulation contributes to gamma oscillation production In vivo and In vitro (Buhl et al., 1998; Hentschke et al., 2007; Liljenstrom and Hasselmo, 1995; Pafundo et al., 2013; Tiesinga et al., 2001). Synchronized gamma band (30-80 Hz) oscillations may be an index of ACh signaling during cognitive tasks, since gamma band power increases in relation to working memory load (Roux et al., 2012) and abnormal gamma oscillations are associated with cognitive deficits (Sun et al., 2012; Uhlhaas and Singer, 2006; Uhlhaas et al., 2011). It has been suggested that high frequency oscillations (above 30 Hz) reflect synchronization of neuronal ensembles that are interacting over short distances in response to primarily sensory processes (Bressler and Freeman, 1980; Ohl et al., 2003).

Whether the electrophysiological results obtained were due exclusively to the loss of cholinergic tone or whether they reflect a compensation or adaption of other brain systems to the loss of cholinergic tone is not clear at the time. Independent of the exact mechanisms of the effects observed, we have demonstrated that loss of cholinergic modulation can influence the output of brain oscillations in this rat model in an active discrimination task. These findings could theoretically contribute to the understanding of the role of cholinergic function in a number of cognitive disorders including: Alzheimer diseases, Parkinson diseases, other cognitive disorders including Down-syndrome, progressive supranuclear palsy, Jakob–Creutzfeldt disease, Korsakoff's syndrome and traumatic brain injury and alcoholism.

5. Conclusions

These studies suggest that ERO energy and phase locking differences can be detected using an active discrimination task in rodents. The data also provide evidence to support the idea that the NBM cholinergic system is involved in maintaining the synchronization/phase resetting of oscillations in different frequencies in response to the presentation of the target stimuli in an active discrimination task.

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Abbreviations

Ach	Acetylcholine
AD	Alzheimer's disease
ANOVA	Analysis of Variance
ChAT	Choline Acetyltransferase
EEG	Electroencephalogram
ERO	Event-related oscillations
ERP	Event-related potentials
Fctz	Frontal Cortex
NBM	nucleus basalis magnocellularis
PLI	phase locking index
ROI	region of interest

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Figure 1. Response to target stimuli. Correct responses (%) to target (top) and time to respond to target stimuli (bottom). (* $p < 0.01$, sham vs NBM lesion)

Figure 2.

ERO energy and phase lock index (PLI) at baseline. Means for all animals pre-lesion ($n=18$) shown from one way ANOVA at each band time point for the target vs non target tone. Tone was compared at 200-500 ms (delta frequency band only) and at N1 (50-200 ms range), P3a (275-325 ms range) and P3b (350-400 ms range) in the theta, alpha, beta and gamma bands. Tone significantly increased ERO energy in the frontal cortex in the beta frequency band in P3a and P3b and gamma frequency band in N1, P3a and P3b. Bottom: Phase locking index (PLI) is significantly increased by tone in alpha and gamma bands at all time points, theta in N1, beta in N1 and P3b. (* $p<0.01$)

Figure 3.

Grand averages of event related oscillations energy color equivalent for sham-operated, and NBM-lesion in the frontal cortex. Each graph depicts a time-frequency representation of ERO energy values in the delta, theta, alpha, beta and gamma bands following the target tone in frontal cortex-central cortex electrode locations. In each graph frequency (Hz) is presented on the Y-axis, time regions of interest on the X-axis (ms) and ERO energy is presented as color equivalents of energy as indicated in the color bar at the bottom of each graph. NBM-lesion produced increases in color equivalents (color equivalents in SHAM and LESION were scaled for visual purposes). Grand averages for A (SHAM) and B (LESION) and the corresponding A' and B' recording at 15 days (15D) after lesion of the NBM shows the change in energy (from baseline).

Figure 4.

NBM-lesion increases ERO energy in the frontal cortex. Estimated marginal mean values from 2 (group) $\times$ 2 (tone) ANOVA for the event-related oscillations energy equivalents in frequency bands (δ , θ , α , β , and γ) of sham operated ($n=8$) and NBM lesioned ($N=10$) subjects at 15 days post lesion. Tone (non target, target) and group (sham, lesion) were compared at 200-500 ms (delta frequency band only) and at N1 (50-200 ms range), P3a (275-325 ms range) and P3b (350-400 ms range) in the theta, alpha, beta and gamma bands. Compared to sham lesions, lesions in NBM significantly increased ERO energy in the

frontal cortex in the delta, theta, alpha and beta frequency band in N1, P3a and P3b. Tone effects were seen in gamma P3a, while tone and a group tone interaction in gamma P3b. Bottom: Phase locking index (PLI) is significantly increased in theta N1 component for NBM lesioned rats. Tone effects were seen in all bands at all time points as well as a group tone interaction in beta P3b. (* $p < 0.01$ group effect, + $p < 0.01$ tone effect, *+ $p < 0.01$ group tone interaction)