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Patterns of electrical activity synchronization in the pregnant rat uterus

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ABSTRACT

Studies of the synchronization of uterine electromyography (EMG) recordings can offer insight into the underlying dynamics of the uterus. Many human studies have shown that synchronization increases throughout pregnancy and at the onset of labor. We investigated whether this phenomenon occurs in other species and if it could thus be generalized. To this end we calculated the nonlinear correlation coefficient (h^2) for 30 uterine EMG channels positioned on the uterus of pregnant Wistar rats using an experimental protocol developed in our laboratory. The results obtained for 16 rats recorded on different gestational days revealed a notable increase in h^2 values throughout pregnancy and at term. These results could improve our understanding of physiological changes in uterine activity and help in enhancing labor detection.

1. Introduction

Uterine electromyography (EMG) is a technique that has been developed for monitoring uterine activity since the 1990s [1–3]. In recent years, uterine EMG has been the subject of many research studies. Studies in both women [4,5] and animals [6–8] have demonstrated that this technique can offer an insight into the progression of pregnancy and the onset of labor. Various temporal and spectral characteristics of uterine EMG signals have been defined for pregnancy and labor [9,10] and their use has been proposed for monitoring pregnancy and detecting labor [5]. The results obtained are encouraging. However, pregnancy monitoring may be improved using nonlinear methods since these methods take into account the nonlinear nature of the signals. A promising technique for this purpose is the nonlinear regression analysis introduced by Pijn and Lopes da Silva [11] in the field of electroencephalograpy (EEG). This approach can be used to study synchronization and to quantify the relationship between signals. Application of this analysis to uterine EMG signals has already provided good results. Terrien et al applied the technique to uterine EMG signals recorded for a monkey during labor [12]. They found higher h^2 values during contractile events compared to baseline h^2 values. Hassan et al investigated the spatial synchronization of uterine electrical activity recorded using a 4×4 electrode grid on the abdomen [13]. The results revealed an increase in the degree of synchronization between contractions throughout pregnancy.

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For application of nonlinear regression analysis to other species, multichannel recordings are essential. In previous studies, Lammers et al used an array of 240 extracellular surface electrodes covering an area of 15 mm × 16 mm [14] to investigate *in vitro* the propagation of slow waves in rat myometrium [15] and rabbit duodenum [16]. However, there have been no studies of the synchronization of recorded signals. In this study, we describe a new *ex vivo* recording protocol developed in the laboratory of Biomechanics and Bio-Engineering at UMR 6600 of the University of Technology of Compiegne to record uterine EMG signals using pin electrodes positioned directly on the uterus of pregnant Wistar rats. All signals are recorded simultaneously and the electrical propagation features can be reconstructed. Such analysis allows detection and investigation of the synchronization of recorded signals.

2. Materials and methods

2.1. Protocol

Approval for this study was granted by the Animal Care Laboratory of the University of Technology of Compiegne. Our animal house is approved under License No. B-60-60159-001 and our experimental protocol was accepted by the Directorate of Veterinary Services.

Our recording protocol was tested on 16 Wistar rats (4 months old) with a timed pregnancy term. Signals were recorded for four rats for every day from 17 to 21 days of pregnancy. All rats were weighed on a biomedical precision balance. Their weights varied from 340 to 460 g. Females in proestrus were caged overnight with males of proven fertility. Vaginal smears were obtained daily and only animals showing three consecutive 4-day cycles were used. Pregnancy was confirmed by the presence of spermatozoa in the vaginal smear the morning after coitus, defined as Day 1 of pregnancy. In our colony, delivery occurs on Day 21 and we used this as the normal gestation period in our study.

The procedures were performed under sterile conditions. Each rat was first anaesthetized with pentobarbital for surgery and recordings. After a mid-abdominal incision was made, the two uterine horns, which can contain an average of five to eight baby rats each, were located. Only one of the uterine horns was chosen for the experiment. Two strings were quickly tied around the end of the selected horn (Fig. 1) at a distance of 5 mm apart to prevent bleeding and release of the baby rats. After cutting the selected uterine horn between the two ties, the horn was excised, keeping the ovarian end attached, and placed in a suitable custom-made support (Fig. 2). The string was attached to a force transducer (range 0–0.5 N). To obtain good extension, the horn was pinned and hooked at the ovarian end. This procedure allowed us to record electrical and mechanical activity simultaneously while the uterine horn was connected to the rat's vascular system.

To record electrical and mechanical activity simultaneously, great care was taken in positioning of the uterine horn and the electrode assembly. A 30-electrode recording array (3 × 10 nickel/tin-plated terminal pins of 0.3 mm in diameter at an inter-electrode distance of 4.5 mm) were gently positioned along the length of the selected horn. Recorded signals were amplified between 0.16 and 256 Hz using BioSemi Active-One amplifiers and sampled at 128 Hz with an anti-aliasing low-pass filter of 64 Hz.

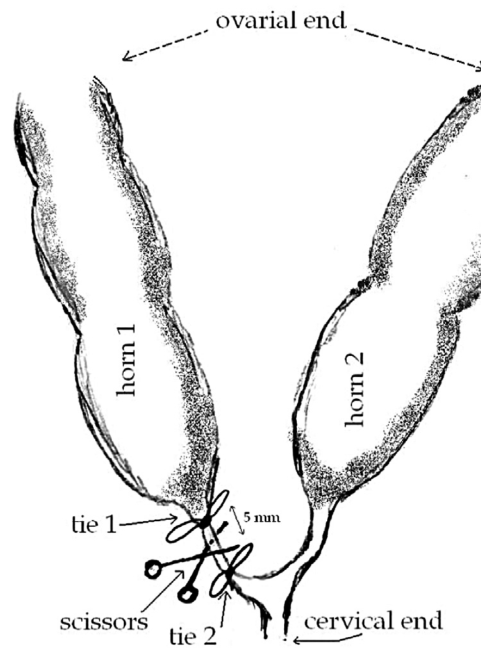

Fig. 1 – Two strings were tied around the end of the uterine horn to prevent bleeding and release of the baby rats.

The signals were digitally filtered to remove ambient 50-Hz noise and reduce the frequency range to 1–40 Hz. Therefore, our *ex vivo* model allows recording of uterine electrical activity while the uterus is still connected to the rat's body.

2.2. Nonlinear regression analysis

Nonlinear regression analysis is a non-parametric method used to quantify and evaluate the dependence of a random process (a time-series signal Y recorded from G_Y, for instance) on another process (signal X recorded from G_x, for instance) from samples (no data model), independent of the type of

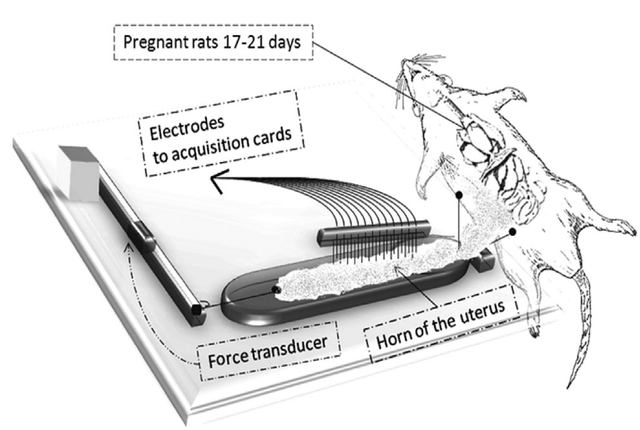

Fig. 2 – Prototype used to record electrical activity with 30 electrode pins.

relationship between the two processes. This method was first used in the field of EEG analysis by Pijn and colleagues [17, 18], who showed that it performed better than methods based on the linear regression or mutual information for analyzing the interdependence of intracerebral EEG signals in an experimental model of generalized epilepsy. The method was then evaluated using a realistic simulation of EEG signals generated by coupled populations of neurons [19]. The correlation ratio h^2 describes the reduction in variance of Y that can be obtained by predicting Y values from X values according to $h^2 =$ (total variance − unexplained variance)/total variance. In practice, the nonlinear correlation coefficient h^2 is estimated from a scatter plot of Y versus X. The values of X are subdivided into bins; for each bin we calculate the X value of the midpoint (p_i) and the average value of Y (q_i) computed for the same bin interval. The regression curve is approximated by connecting the resulting points (p_i, q_i) by straight-line segments [20]. Then the nonlinear correlation coefficient between the X and Y signals is calculated as

$$h^2_{Y/X} = \frac{\sum_{K=1}^{N} X(k)^2 - \sum_{K=1}^{N} (Y(k) - f(X_i))}{\sum_{K=1}^{N} Y(k)^2},$$

where $f(X_i)$ is the linear piecewise approximation of the nonlinear regression curve. The values of h^2 range between 0 (X and Y are independent) and 1 (Y is determined by X). One of the advantages of nonlinear regression is that it can be applied to signals independently of whether their relationship is linear or not.

Another interesting property of the nonlinear correlation coefficient h^2 is that it is asymmetric: the h^2 value from signal X to signal Y differs from the value from signal Y to X. Wendling et al used this property and proposed a new parameter they called the direction index to show the direction of the dependence between X and Y [18].

3. Results

To evaluate our method, simultaneous recordings of electrical and mechanical activity were made for 16 pregnant Wistar rats on different gestational days (Days 17, 19, 20, and 21) using the *ex vivo* protocol described above.

Fig. 3 shows action potential bursts obtained from the three columns of electrodes (30 electrodes), oriented in the longitudinal axis of the uterine horn, for one uterine contraction recorded with the new *ex vivo* protocol.

The h^2 values were computed pairwise using 900 possible pairs for the 30 monopolar signals recorded from the *ex vivo*

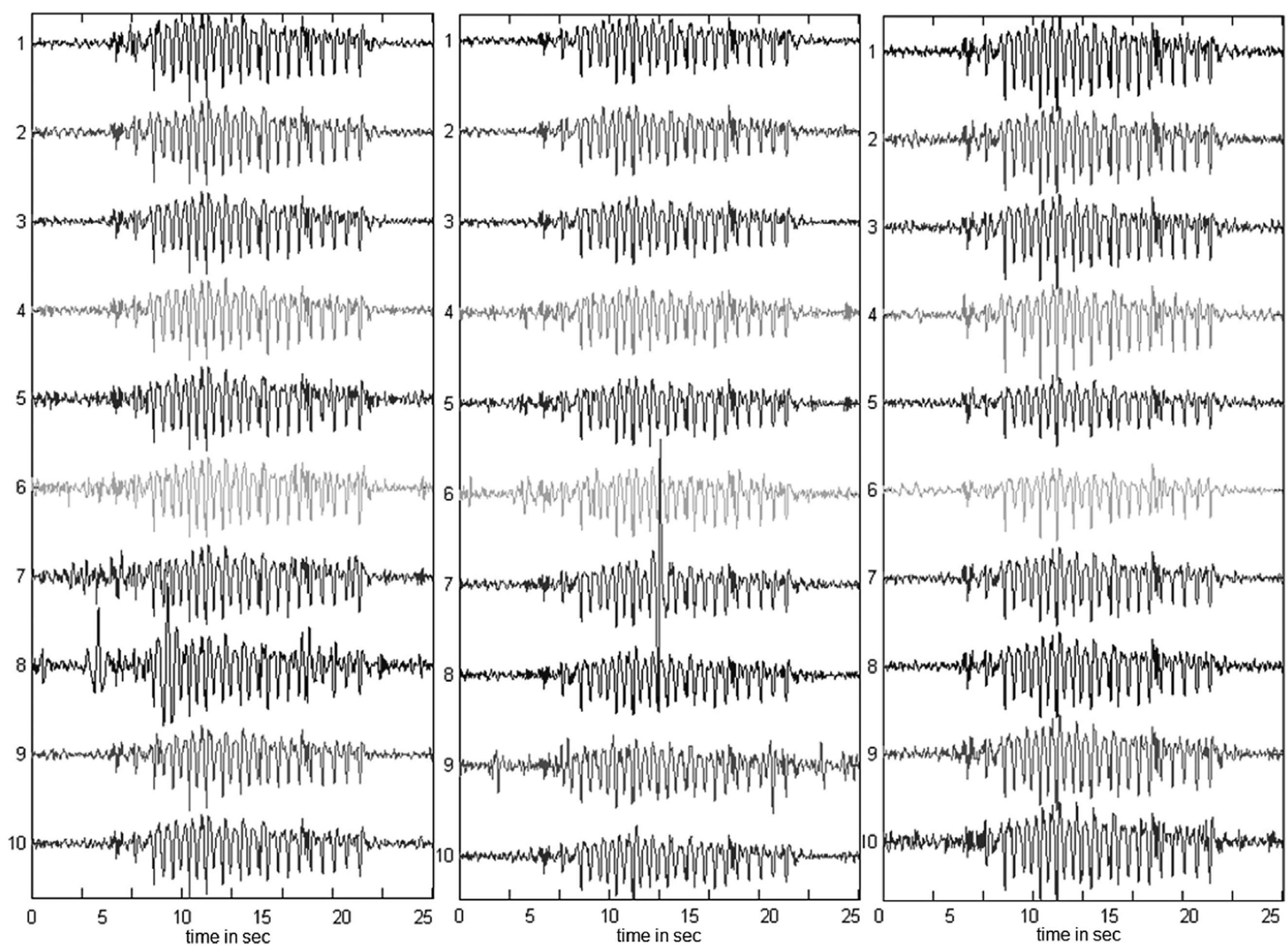

Fig. 3 – Action potential bursts registered during a contraction in three columns of electrodes along the longitudinal axis on Day 17 of pregnancy.

uterus. We represent the nonlinear correlation values using color-coded matrices. Therefore, all h^2 values calculated over one contraction are represented in one correlation matrix [13]. First, we studied the evolution of the synchronization throughout pregnancy by plotting the color-coded matrices and their mean values against the number of gestation days and at the onset of labor. To this end, we first calculated the mean h^2 value over the matrix related to each recorded contraction. Then we calculated the mean of these values over all contractions associated with each pregnancy timepoint. Fig. 4 shows the results. It is clear that there was an increase in h^2 values during pregnancy (Fig. 4A–C) and at term (Fig. 4D). The h^2 matrices have low values over almost all the channels for the Day 17 contractions (Fig. 4A) and high values for contractions on Day 21 (Fig. 4D), which indicates a lower degree of synchronization of uterine cells on Day 17 of pregnancy than in labor. Furthermore, the mean h^2 matrix value increased from 0.86 ± 0.1 on Day 17 to 0.98 ± 0.07 on Day 21. Fig. 5 and Table 1 present the mean values at each timepoint and the results of Student's t test applied to the data.

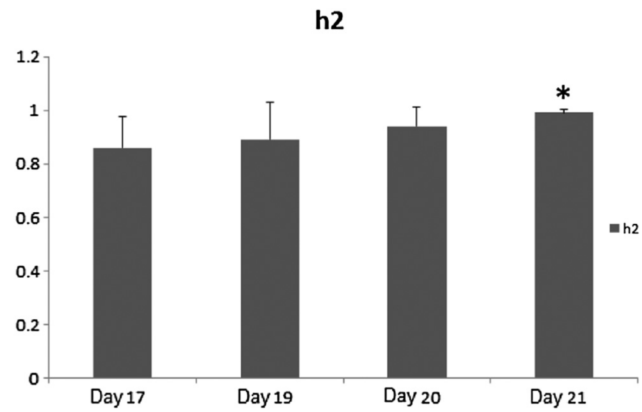

Fig. 5 – Change in the mean h^2 matrix value from Day 17 to Day 21. *Significant difference ($p < 0.05$).

4. Discussion

We used nonlinear regression analysis to study the synchronization between uterine EMG signals recorded from the uterus of Wistar rats. A new protocol was developed to record uterine electrical activity through 30 electrode pins in an *ex vivo* uterus. We analyzed data recorded for 16 Wistar rats on different gestational days. The results show that synchronization increases during pregnancy. There was a notable increase for all h^2 matrices during pregnancy and at the onset of labor. This confirms the results obtained in

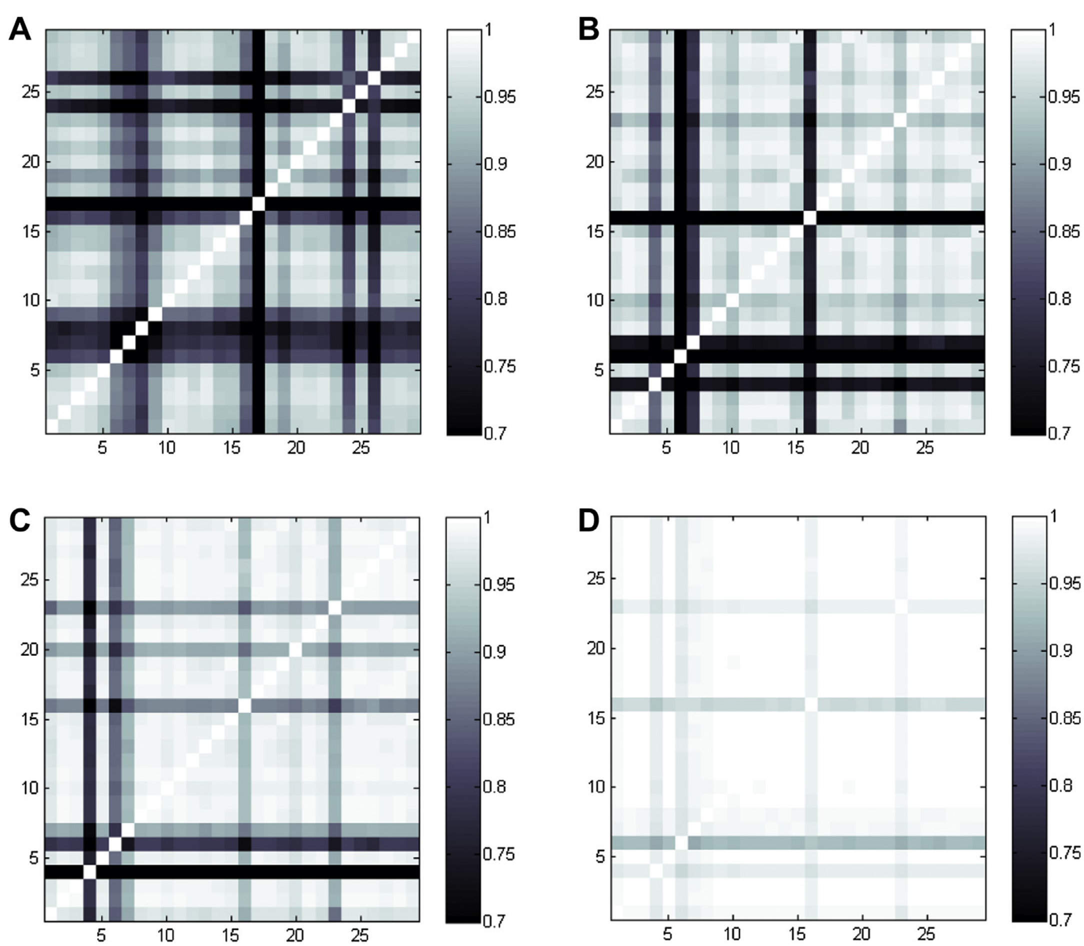

Fig. 4 – Color-coded matrices representing h^2 for various gestational timepoints: (A) Day 17, (B) Day 19, (C) Day 20 and (D) Day 21.

Table 1 – Results for Student's t test applied to the mean value at each gestational timepoint.

	D17–D19	D17–D20	D17–D21	D19–D20	D19–D21	D20–D21
p	0.0572	0.0012*	0.00411*	0.2174	0.019*	0.0013*

*Significant difference between the two timepoints considered.

humans [13] but we used an electrode grid covering the majority of the uterus volume. We analyzed statistical differences between gestational timepoints using Student's *t* test (Table 1). Although there was no systematic significant difference between two consecutive points during pregnancy, there was a significant difference in mean h^2 between values recorded during labor and any other value recorded during pregnancy. Therefore, our study shows that nonlinear regression analysis represents a powerful tool for distinguishing between pregnancy and labor using uterine EMG signals recorded for a living pregnant rat.

5. Conclusion

We described a new protocol for recording uterine electrical activity in pregnant rats. The signals recorded for 16 pregnant Wistar rats on different gestational days were subjected to nonlinear regression analysis. The results revealed more synchronized activity during labor than in pregnancy. The difference in mean h^2 values between pregnancy and labor was significant. This recording technique can be used to improve our understanding of physiological changes during pregnancy by analyzing electrical activity of the uterus while it is still connected to the body by giving uterotonic or tocolytic agents to living pregnant rats.

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