
CHAOTIC, FRACTAL, AND NONLINEAR SIGNAL PROCESSING

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Nonlinear Analysis and Prediction of Pulsatile Hormone Secretion

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Abstract. Pulsatile hormone secretion is observed in almost every hormonal system. The frequency of episodic hormone release ranges from approximately 10 to 100 pulses in 24 hours. This temporal mode of secretion is an important feature of intercellular information transfer in addition to a dose-response dependent regulation. It has been demonstrated in a number of experiments that changes in the temporal pattern of pulsatile hormone secretion specifically regulate cellular and organ function and structure. Recent evidence links osteoporosis, a disease characterized by loss of bone mass and structure, to changes in the dynamics of pulsatile parathyroid hormone (PTH) secretion. In our study we applied nonlinear and linear time series prediction to characterize the secretory dynamics of PTH in both healthy human subjects and patients with osteoporosis. Osteoporotic patients appear to lack periods of high predictability found in normal humans. In contrast to patients with osteoporosis patients with hyperparathyroidism, a condition which despite sometimes reduced bone mass has a preserved bone architecture, show periods of high predictability of PTH secretion. Using stochastic surrogate data sets which match certain statistical properties of the original time series significant nonlinear determinism could be found for the PTH time series of a group of healthy subjects. Using classical nonlinear analytical techniques we could demonstrate that the irregular pattern of pulsatile PTH secretion in healthy men exhibits characteristics of deterministic chaos. Pulsatile secretion of PTH in healthy subjects seems to be a first example of nonlinear determinism in an apparently irregular hormonal rhythm in human physiology.

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INTRODUCTION

Hormonal systems are regulated dynamically (1). There has been growing evidence in recent years that oscillations of the plasma concentration of parathyroid hormone (PTH) on short time scales play an important role in the physiological bone remodeling process which ensures a balance of bone resorption and bone formation (2-4). PTH in healthy humans is secreted in an episodic manner in distinct pulses with a frequency ranging from 1 pulse/hour for large pulses and 1 pulse/10 minutes for smaller pulses (2). In animal experiments intermittent administration of PTH increases bone mass and may enhance normal bone connectivity, whereas continuous infusion of the same dose leads to a dramatic loss of bone mass and structure (5-7). As these changes occur in osteoporotic patients, it is conceivable that this disease may also be caused by a disruption of the normal temporal dynamics of episodic PTH secretion. On the other hand patients with hyperparathyroidism have a well preserved trabecular bone structure (8). These results suggest that the temporal pattern of PTH secretion in osteoporotic patients, patients with hyperparathyroidism, and healthy controls would be different. And in fact these differences were found in recent studies in healthy subjects, patients with osteoporosis, and hyperparathyroidism by drawing blood every two minutes over several hours (2, 9).

In recent work the technique of time series prediction has been used to effectively characterize irregular complex time series and separate nonlinear deterministic (chaotic) from different forms of stochastic behavior (10-12). To reliably discriminate between nonlinear determinism and stochastic dynamics in a time series the technique of surrogate random data has been used (13, 14). Predictive models have shown to be particularly effective when applied to short time series containing on the order of a couple of hundred data points (11, 12). Differences in the system dynamics are reflected in different degrees of predictability. This approach has been applied to the classification of various biological time series (15) as for example EEG (electroencephalogram) data (16), ECG (electrocardiogram) data (17) and experimental data from simple neuronal circuits (18, 19).

Here we apply the technique of time series prediction in conjunction with the technique of generating random surrogate data for classifying and characterizing the dynamics of PTH secretion in nine healthy subjects, six patients with osteoporosis, and four patients with hyperparathyroidism. In a recent study using standard nonlinear analytical techniques such as the calculation of the correlation dimension (20), the Lyapunov exponents (21) and Kolmogorov entropy (22) compared with surrogate random data we could demonstrate nonlinear determinism (chaos) in three 21h viz. 24h PTH time series from healthy subjects (23). These PTH time series (Fig. 1) exhibit an irregular temporal pattern of secretion corresponding to broadband power spectra (Fig. 2).

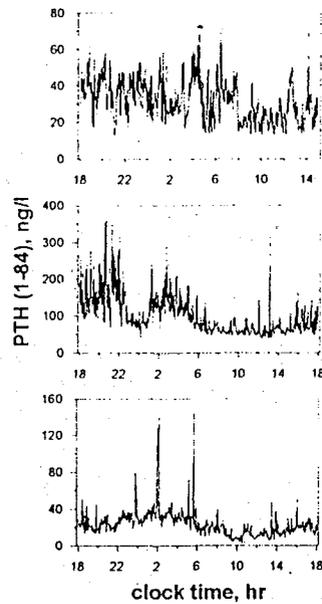


FIGURE 1. Time series of parathyroid hormone (PTH)-(1-84) plasma concentration in 3 healthy male subjects.

METHODS

Subjects

Twelve healthy men (aged 24-42 years), three women with postmenopausal osteoporosis (aged 55-62 years), three men with idiopathic osteoporosis (aged 31-42 years), one male (56 years), and three female patients (aged 60-65 years) with primary hyperparathyroidism took part in this study. The studies reported here were approved by the local Committee on Medical Ethics, and all subjects gave their informed written consent. Some of the subjects in this study as well as the details on design and measurements can be found in detail earlier (2, 9, 23).

Hormone analysis

In three of the healthy subjects, blood samples were taken every two minutes over an extended period (21 hours for one subject and 24 hours for the other two) for

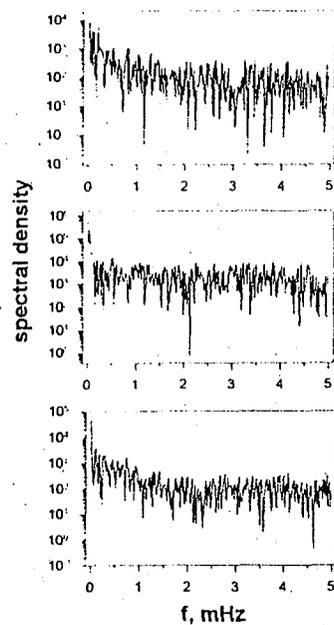


FIGURE 2. Fourier power spectrum of PTH concentration time series from Fig. 1.

determining the PTH plasma concentration. In the remaining 9 healthy subjects, 6 osteoporotic patients, and 4 patients with hyperparathyroidism PTH serum concentrations were also measured at two minute intervals, but over much shorter periods of time (3.5 to 9 hours) as shown in Figure 3. All PTH concentrations were measured in duplicate using either a two-site chemiluminometric (sandwich) immunoassay (Magic Lite Intact PTH, Ciba-Corning Diagnostics Corporation, Medfield, MA; intra-assay coefficient of variation (CV) 3.4%, inter-assay CV 4.3%) or an intact PTH immunoradiometric assay (Nicols, San Juan Capistrano, CA; intra-assay CV 5.1%; inter-assay CV 7.8%). Blood samples (1 ml) were drawn via a central venous catheter. Throughout the study, the subjects rested in bed.

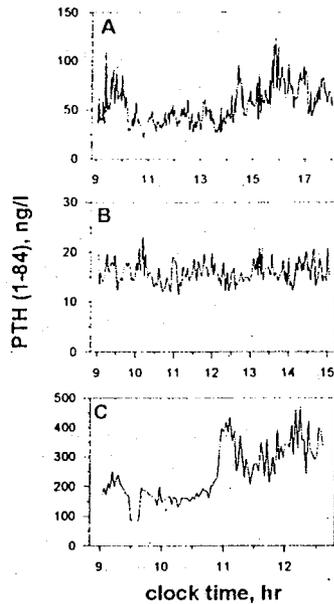


FIGURE 3. Three representative PTH plasma concentration time series in a A) healthy subject, B) osteoporotic patient, and C) patient with hyperparathyroidism.

Time series prediction

Neural network approaches have been demonstrated to be powerful tools for time series prediction (24). Such a neural network model predicts the next value $x(t_i)$ of a time series from a number of previous values:

$$x(t_i) = f(x(t_{i-1}), x(t_{i-2}), \dots, x(t_{i-m})) + \epsilon_i \quad (1)$$

where $x(t_{i-1})$ is the value at time t_{i-1} , m is the number of previous values used for prediction, and ϵ_i represents noise or fitting error. The model f is selected by minimizing some measure of misfit (such as mean square error) in a set of training examples selected from the samples of the time series.

In our study we used feedforward networks with nonlinear (sigmoidal) activation functions as well as linear activation functions (equivalent to autoregressive

models) to predict future values of the time series of PTH serum concentrations. This form of a predictor was chosen because it is relatively easy to control over-fitting using regularization functions and cross-validation. Such connectionistic models have shown to be effective to predict short (noisy) time series (24-27).

The neural networks were trained to predict one time step into the future. To predict multiple time steps ahead the value predicted one time step ahead was iterated by feeding it back into the input of the network. If the predicted value one step into the future is:

$$\hat{x}(t) = f(x(t_{i-1}), x(t_{i-2}), \dots, x(t_{i-m})) \quad (2)$$

then the predicted value two time steps ahead is defined as:

$$\hat{x}(t_{i+1}) = f(\hat{x}(t), x(t_{i-1}), \dots, x(t_{i-m+1})) \quad (3)$$

To predict any desired number of time steps into the future this procedure can be repeated. The prediction error was estimated using the *average relative variance (arv)*:

$$arv = \frac{\langle (x_i - \hat{x}_i)^2 \rangle}{\sigma^2(x_i)} \quad (4)$$

where the angle brackets denote the over all mean and $\sigma^2(x_i)$ denotes the variance of the indexed variable.

To improve the signal to noise ratio in our experimental data the raw PTH time series were filtered prior to the connectionist approach using an acausal filter (28) which has been shown not to affect certain nonlinear measures as for example the correlation dimension.

Training and testing of predictive models

A large variety of different network architectures was explored, applying feedforward networks with linear as well as nonlinear (sigmoidal) activation functions with 5 to 15 input units, 0 to 5 hidden units, and 1 output unit. A regularization technique (26) was used to control overfitting to the training data, with the weight assigned to the regularization term chosen by cross validation. The value for the regularization term varied from 10^{-5} for the nonlinear networks to 10^{-10} for the linear networks. The weights were updated by a conjugate gradient descent method. (For a linear model, this fitting technique is formally equivalent to a least-squares linear fit.)

Networks for the healthy group were trained using pooled time series data from the three healthy male subjects whose PTH serum concentrations were measured every 2 minutes over extended periods of 21 viz. 24 hours. Predictive networks for the patients with osteoporosis viz. hyperparathyroidism were trained using a "leave one out" technique where all data from the patient group besides the time series to be predicted were used for training. The performance of a large variety of network architectures as described above was compared using the *arv* as measure for the prediction error.

Because of their predictive performance a nonlinear 15 input units, 5 hidden units and 1 output unit network with sigmoidal activation functions and a 15 input units and 1 output unit linear network were used for the simulations discussed in the remainder of this paper. After training with pooled data within each subject viz. patient group the network was used for predicting each of the time series from the three groups.

To measure the degree of predictability the prediction error *arv* was computed for each of the nineteen test subjects as a function of the number of steps predicted into the future as well as for ten surrogate time series generated by two different procedures as explained below. The number of time steps for each time series where the *arv* reached a value of 0.5 was recorded. This cut-off criterion ensures that the predictive model is still useful for predicting the system dynamics whereas an *arv* of 1.0 may be achieved by always guessing the mean of the time series as the predicted value.

Surrogate data

We generated surrogate data as statistical controls for our experimental data to test the null hypothesis that the predictive results can be explained by a stochastic process. Surrogate data are randomized versions of the original data sets preserving certain statistical features of their originals. For each original time series we generated ten surrogate data time series by two different procedures as proposed by Theiler *et al.* (13).

Phase randomized surrogate data

In this procedure the original time series is Fourier transformed to obtain phase and amplitude spectra. The phases are randomized using i.i.d. (independent identically distributed) random numbers from an $[0, 2\pi]$ interval and after transforming back to the time domain one gets a randomized version of the original time series which preserves the power spectrum, the mean, the standard

deviation, and the autocorrelation. This sort of surrogate data is used to test the null hypothesis that the results can be explained by using data from a Gaussian linear stochastic process. Other types of surrogate data are necessary since this procedure does not preserve the histogram of the signal amplitudes.

Gaussian scaled surrogate data

Gaussian scaled surrogate data are generated by shuffling the original data in such a way that the amplitude distribution of the original is preserved and that the power spectrum remains very similar but not identical as in the case of the phase randomized surrogates. Using this sort of surrogates we can test the null hypothesis that our results can be explained by testing data from a nonlinear static transformation of a linear stochastic process.

Statistical significance

To measure the amount of deterministic structure that might have been destroyed by either of the randomization techniques we used the significance level S , defined as

$$S = \frac{\langle T_{\text{surrogate}} \rangle - T_{\text{original}}}{\sigma} \quad (5)$$

where T is the prediction time step where the *arv* exceeds a value of 0.5. The angle brackets denote the over all mean of ten surrogate time series generated for each original by either of the two methods and σ is the standard deviation of the results obtained from the surrogates.

RESULTS

Using a large variety of linear as well as nonlinear neural network predictors we found a linear increase of the prediction error *arv* within the osteoporotic patients whereas the healthy subjects and the patients with hyperparathyroidism showed a saturation of the *arv* on a much lower level than the osteoporotic group. Figure 4 displays the predictive results of a 15 input units, 5 hidden units and 1 output unit network with sigmoidal activation functions and a 15 input unit and 1 output unit network with linear activation functions. However, the performance between linear and nonlinear predictors in general was not significantly different within each group (Fig. 5).

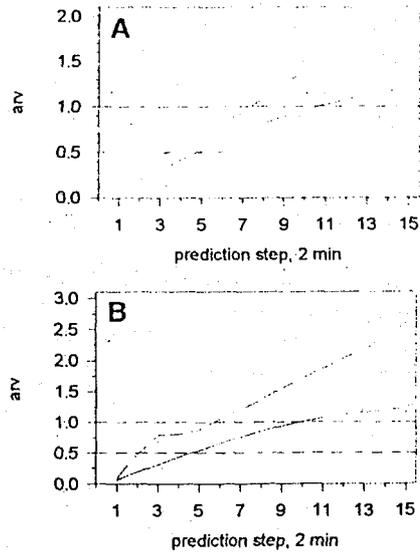


FIGURE 4. Prediction error arv versus prediction step (2 min) for normal subjects ($n=9$, solid line), patients with osteoporosis ($n=6$, dashed line), and patients with hyperparathyroidism ($n=4$, dotted line). **A)** 15 input units, 5 hidden units, and 1 output unit neural network with sigmoidal activation functions. **B)** 15 input units, 1 output unit neural network with linear activation functions.

To test for nonlinear determinism we evaluated the predictive ability of our neural network on 10 phase randomized and 10 Gaussian scaled surrogate data sets. We could demonstrate a significance level S between 1.9 and 2.3 for the predictability of time series from all three groups compared to their corresponding Gaussian scaled surrogate data using a nonlinear network (Table 1). Only the normal subjects showed a relatively low significance level S of 1.0 for the difference between the predictability of the original time series and the corresponding phase randomized data sets whereas the osteoporotic patients and hyperparathyroid patients did not show a significant difference. Using a linear predictor for comparing its predictive ability for the original data with that for the surrogates we found similar results (Table 2). A significant difference from both types of surrogate data could be found only for the normal subjects with significance levels S above 2.0. Although the predictability in some of the osteoporotic patients and

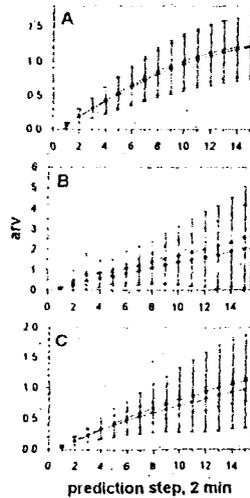


FIGURE 5. Predictability of a nonlinear 15 input unit, 5 hidden unit, and 1 output unit neural network(■) versus linear 15 input unit and 1 output unit neural network(●). Mean $arv \pm$ standard deviation. A) normal subjects, B) patients with osteoporosis C) patients with hyperparathyroidism.

patients with hyperparathyroidism was above a significance level of 2 (more than two σ different from the surrogates), no significant difference could be found for both patient groups.

Whereas some time series from the osteoporotic and the normal group had a significantly higher predictability than their phase randomized counterparts such behavior could not be demonstrated for any of the hyperparathyroid patients. Figure 6 displays the prediction error arv versus the prediction step for the original time series and both types of surrogate data from one healthy subject viz. patient of each group using the 15 input units sigmoidal neural network. The prediction error for the normal subject lies below the scatter of both types of surrogates for most of the time steps whereas the prediction error for the osteoporotic patient keeps within the scatter of both types of surrogate data sets for all prediction time steps. In the hyperparathyroid patient we find an almost undistinguishable prediction error arv for the original time series from that of the phase randomized surrogate data. However, the arv of the original data lies outside the scatter of the Gaussian scaled surrogates for all prediction steps.

Using a linear network with the same length of the input window (Fig. 7) the difference of the prediction error arv between the surrogate scatter and the original

TABLE 1. Prediction step (in 2min) where the *arv* exceeds 0.5 (15-5-1 nonlinear network). Statistics using the significance level S as described above.

group	original (I)	phase surrogates (II)	Gaussian surrogates (III)	I vs. II	I vs. III
normals	6.6±3.2	6.0±2.7	4.7±1.7	1.0	2.3
osteoporosis	4.6±2.6	5.1±2.6	3.1±0.6	0.1	1.9
hyperparathyroidism	8.0±4.1	6.5±2.5	2.2±0.5	0.5	2.2

TABLE 2. Prediction step (in 2min) where *the arv* exceeds 0.5 (15-1 linear network). Statistics using the significance level S as described above.

group	original (I)	phase surrogates (II)	Gaussian surrogates (III)	I vs. II	I vs. III
normals	6.2±2.7	4.9±3.0	4.0±2.2	2.1	2.9
osteoporosis	3.6±1.5	4.2±2.5	2.7±0.9	0.6	1.6
hyperparathyroidism	7.3±2.6	6.9±2.7	3.9±1.7	0.2	1.8

in the normal subject decreases and the osteoporotic subject displays a clearly reduced predictability than using a nonlinear predictor. The prediction error of the hyperparathyroid subject is not distinguishable from both types of surrogate data.

DISCUSSION

We found no evidence of a highly predictable saturating component in any of the osteoporotic time series using our predictive approach. Although the hyperparathyroid patients demonstrated high predictability similar to that of the normal subjects it was only significantly different from their corresponding Gaussian surrogate data sets using a nonlinear predictor. The osteoporotic group did not show a significant difference between the predictability of the original data and both types of surrogates using linear as well as nonlinear predictors.

Confirming results from a former study on nonlinear determinism in extended time series of PTH concentration in healthy subjects we could observe significant nonlinear determinism in nine shorter time series of healthy subjects using linear and nonlinear time series prediction and statistical tests against phase randomized as well as Gaussian scaled surrogate data sets. This suggests that osteoporotic patients and patients with hyperparathyroidism have lost at least in part nonlinear

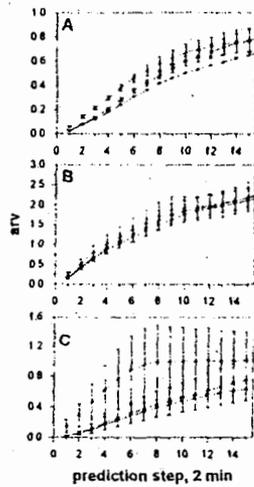


FIGURE 6. Predictability of a 15 input units, 5 hidden units, and 1 output unit nonlinear neural network for original time series (●) and mean \pm standard deviation of 10 phase randomized surrogate data sets (■) viz. 10 Gaussian scaled surrogate data sets (▲). A) normal subject, B) patient with osteoporosis, C) patient with hyperparathyroidism.

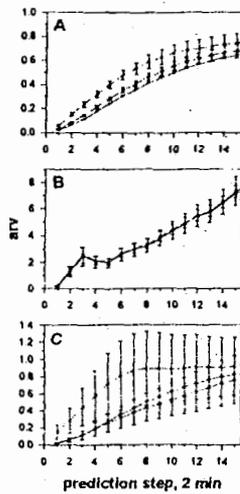


FIGURE 7. Predictability of a 15 input units and 1 output unit linear neural network for original time series (●) and mean \pm standard deviation of 10 phase randomized surrogate data sets (■) viz. 10 Gaussian scaled surrogate data sets (▲). A) normal subject, B) patient with osteoporosis, C) patient with hyperparathyroidism.

deterministic structure in their secretory dynamics of PTH.

The major dynamic regulator for the normal coupling of bone resorption and formation in the bone remodeling process appears to be found in the predictability of the secretory dynamics of PTH.

However, a detailed understanding of the secretory dynamics of PTH and bone anabolic properties is still lacking. Our data provide first evidence that states of high predictability are a regular finding in subjects with normal bone mass and metabolism as well as patients with hyperparathyroidism both of which have a preserved normal bone structure.

Consequently, if a high rate of change of PTH concentration is the significant factor in maintaining normal bone remodeling, frequent application of PTH with large doses may be even more effective than daily injections as are used in current treatment protocols of osteoporosis. Application of large pulses of PTH on an hourly scale might be feasible with the use of a hormone pump, an important field for future investigation.

We found no significant difference in the predictive ability of linear and nonlinear networks for the PTH concentration time series from all groups. However, using classical nonlinear analysis, we have seen evidence for nonlinear determinism and conceivably low-dimensional deterministic chaos in the irregular pattern of PTH secretion in healthy human subjects (23). These results are confirmed by comparing time series prediction of the original data with phase randomized as well as Gaussian scaled surrogate data. Our results are also in accordance with the findings of Blinowska and Malinowski (22) who demonstrated that the irregular nonlinear EEG signal could be predicted equally well or even better by a linear autoregressive model than by the nonlinear prediction model proposed by Sugihara and May (20).

These results differ from others who have compared linear and nonlinear predictive models. This may be due to the relatively small amount of data available in our study (11, 24, 25).

One limitation in our studies is the time period over which PTH concentrations can be measured. Longer periods may only be performed by an online biosensor for PTH to avoid the blood loss due to frequent blood sampling. Such a biosensor for PTH is not yet available although biosensors containing biological receptors such as the nicotinic acetylcholine receptor (29-31) and the *L*-glutamate receptor (32) have been developed.

Time series prediction has been established as a valid tool for the characterization and classification of the dynamics in a variety of biological systems. Using the

technique of surrogate data null hypothesis can be constructed to statistically distinguish between stochastic and deterministic behavior in the dynamics and gain new insight into the physiological regulation of episodic hormone secretion in cases where simple linear methods such as computing the mean value of a time series or the power spectrum might fail to distinguish normal from disturbed patterns. Our results suggest that the PTH secretory pattern in healthy subjects is an example of nonlinear determinism which seems to be lost in part in two bone diseases, osteoporosis and hyperparathyroidism.

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