2021 43rd Annual International Conference of the
IEEE Engineering in Medicine & Biology Society (EMBC)
Oct 31 - Nov 4, 2021. Virtual Conference

Dynamical Characteristics of Wild-Type Mouse Spontaneous Pupillary Fluctuations*

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Abstract— **Spontaneous pupil size fluctuations in humans and mouse models are noninvasively measured data that can be used for early detection of neurodevelopmental spectrum disorders. While highly valuable in such applied studies, pupillometry dynamics and dynamical characteristics have not been fully investigated, although their understanding may potentially lead to the discovery of new information, which cannot be readily uncovered by conventional methods. Properties of pupillometry dynamics, such as determinism, were previously investigated for healthy human subjects; however, the dynamical characteristics of pupillometry data in mouse models, and whether they are similar to those of human subjects, remain largely unknown. Therefore, it is necessary to establish a thorough understanding of the dynamical properties of mouse pupillometry dynamics and to clarify whether it is similar to that of humans. In this study, dynamical pupillometry characteristics from 115 wild-type mouse datasets were investigated by methods of nonlinear time series analysis. Results clearly demonstrated a strong underlying determinism in the investigated data. Additionally, the data's trajectory divergence rate and predictability were estimated.**

I. Introduction

Spontaneous pupil size fluctuations are controlled by the autonomous nervous system. Even under constant conditions pupil size fluctuates and produces complex, seemingly random dynamics [1,2]. Pupil size fluctuations can be used to track changes in mental states and behavioral performance [3]. Pupillometry data of both human subjects and animal models have been utilized in numerous applied studies [1-6], including those on neurodevelopmental disorders [4,5]. This makes a thorough understanding of the dynamics of pupillometry an essential task.

The activity of the brain is highly fluctuating even at the resting state. This intrinsic brain fluctuation may influence the control system of the pupil to produce spontaneous pupillary fluctuations in the time series waveform that possesses a great complexity. Yet, while there are numerous applied studies involving pupil size fluctuations, there seem to be few that investigate its dynamics and properties in detail. In [1,2] pupillometry dynamics from *healthy human subjects* was investigated by methods of nonlinear time series analysis, which have been found useful in numerous studies on the dynamics of complex biological signals [7]. In [1,2] the applied analysis included the time-delay-reconstruction method, the method of surrogate data, and calculation of correlation dimension, the largest Lyapunov exponent, recurrence plot, recurrence rates and determinism, which are recurrence plot quantification analysis measures. As a result, the determinism of the healthy human subjects' data was reported in both studies, and, additionally, in [2] its dynamics was claimed to be deterministic chaos.

Spontaneous oscillations of pupil size in mouse models have been taken as a noninvasive biomarker to address such important and challenging problem as the early detection of neurodevelopmental disorders like autism [5]. Full understanding of the underlying pupillometry dynamics and the extent to which their properties are similar across species may provide new valuable information that could not be uncovered by previously applied analysis methods, potentially yielding better results in existing studies. While some attention has been paid to human pupillometry dynamics as above, practically no studies have investigated the pupillometry dynamics of mice, which can be experimentally manipulated in preclinical models. Thus, a detailed investigation of mouse pupillometry dynamical characteristics is needed.

Pupillometry data from wild-type (WT) mice are used as a control for comparison with diseased mouse models [5]. So, in this study, we aimed to confirm whether the WT mouse pupillometry data have dynamical characteristics similar to those of healthy human subjects, whose dynamics were found to be deterministic and even claimed to be chaotic [1,2]. Presence of 'determinism' implies that the dynamical system is determined by a certain rule and its evolution is defined by the current state and past information, making it one of the defining properties of deterministic chaos. However, it is a very challenging task to prove that experimentally measured pupillometric data have chaotic dynamics. So, in this study, besides the determinism criterion, we estimated two more important properties of complex dynamical systems, namely trajectory divergence and predictability, which can provide a clue on the underlying pupillometry dynamics. The presence of trajectory divergence as well as the possibility to obtain short-term predictions, i.e. forecast process state in short-time (but not long-time) evolution, are important properties of a chaotic dynamical system. Thus, the determinism, trajectory divergence, and predictability of collected WT data [5] were

*Research is partially supported by JSPS KAKENHI Grant Number JP19K14589 to N.S.; HFSP-CDF-2015C (Human Frontiers Science Program -Cross-Disciplinary fellowship) to P.A.; Italian Association of Rett Syndrome (AIRETT) and Hock Tan & Lisa Yang Center for Autism Research Harvard Medical School to M.F.; CIFAR, Bezos and JPB Foundations and JSPS KAKENHI Grant Number 20H05920 to T.K.H; JSPS KAKENHI Grant Number JP20H05921, AMED under Grant Number JP20dm0307009, and JST Moonshot R&D Grant Number JPMJMS2021to K.A.

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carefully tested by multiple methods of nonlinear time series analysis.

II. Materials and Methods

A. Data

Here, spontaneous pupillary fluctuation data recorded by an infrared camera with 50 Hz sampling frequency from 115 WT mice were used. Awake mice were head-fixed but freely moving on a circular treadmill under dim light conditions (50 lux) to allow pupil fluctuations [5]. Pupil size data were collected over 30-minute periods by an infrared camera aimed at the right eye. Data included a variety of age groups from postnatal day P20 to P369. Animal care and experimental procedures were performed in accordance with protocols approved by the Boston Children's Hospital Institutional Animal Care and Use Committee [5]. Detailed information on the experimental procedure can be found in [5].

Data were preprocessed by a low-pass filter with a passband frequency of 6 Hz before the analysis. An example of the resulting pupillometry time series is shown in Fig. 1.

B. Analysis

Methods of nonlinear time series analysis were applied to the WT mouse pupillometry data to unveil its dynamical properties. Most of the methods of nonlinear time series analysis require complete state-space information of the process dynamics. However, for the experimentally measured data often only a single variable observation is all that is available. The method of time-delay reconstruction [7] allows one to obtain possible dynamics of the process in m-dimensional phase space based on single variable observation, $x(t)$. Therefore, first, the possible dynamics was reconstructed in the phase space by the time-delay embedding technique.

To avoid reliance on a single measure to test pupillometry dynamics properties, multiple tests were applied to the reconstructed trajectory. The Wayland test translation error (WTE) [8], which allows to estimate determinism of the data as well as provides an estimate on the level of the noise present in the data, was calculated. Then, the recurrence plot (RP) method [9], which utilizes neighborhood relations to visualize m-dimensional system dynamics as a 2-dimensional binary image, was applied. Depending on the type of system's dynamics RP has a unique pattern that qualitatively characterizes a dynamical system. As the RP provides only qualitative characteristics of investigated data, the recurrence quantification analysis (RQA) [9] was performed. RQA quantifies underlying structures in RP, and, therefore, allows one to go beyond RP image-based qualitative results. Then the largest Lyapunov exponent (LLE), which provides qualitative and quantitative characteristics of the dynamical system, was calculated. Qualitatively, a positive LLE implies the presence of trajectory divergence and is recognized as a hallmark of chaos. Quantitatively the value of the LLE measures the trajectories divergence rate. As the LLE reflects important properties of a dynamical system, it is one of the methods of nonlinear time series analysis frequently used in applied studies. In this study, the LLEs were calculated by the Rosenstein et al. method [10]. Finally, the deterministic nonlinear prediction method was applied to estimate data's predictability. Presence of the short-term predictability can be recognized as a sign of data determinism [7]. Additionally, the method of surrogate data [11] was applied to the LLE and prediction results to address the issue of the noise contamination in the experimental data.

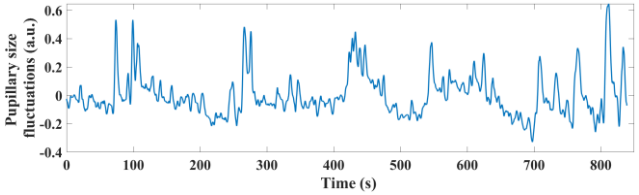

Figure 1. An example of preprocessed wild-type mouse spontaneous pupillary fluctuations time series.

III. Results

A. Time-Delay Reconstruction

The time-delay-reconstructed trajectory was calculated for all datasets. The dimension necessary for the reconstruction was estimated by the false nearest neighbors method [7]. The minimum embedding dimension was estimated as m=4 for 22 datasets and as m=3 for the rest of the datasets; therefore, the reconstruction dimension was chosen as m=4 for further calculations. The value of time delay, τ, applied in time-delay reconstruction was chosen as the time when the time series autocorrelation function falls below (1-1/e). Figure 2 demonstrates an example of the time-delay-reconstructed trajectory corresponding to the time series shown in Fig. 1.

B. Wayland Test Translation Error (WTE)

Figure 3 demonstrates the distribution of WTEs calculated for all datasets. The WTE values close to 0 indicate strong underlying determinism in the data. In contrast, a stochastic process, such as colored noise, produces a WTE value close to 0.5 [12], and an uncorrelated random process, such as white noise, produces a WTE value close to 1. Although there is no clear WTE-value boundary between deterministic and stochastic processes, in previous studies it was discussed [12,13] that WTE values significantly larger than 0, but less than 0.5 can be produced by a deterministic process with a considerable level of noise contamination.

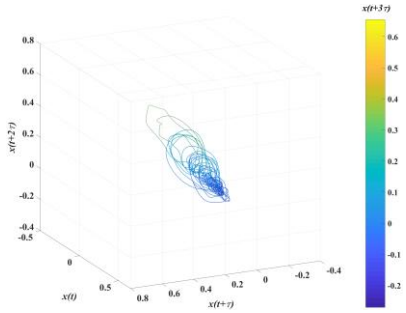

Figure 2. An example of pupillometry time-delay-reconstructed trajectory in the 4-dimensional phase space.

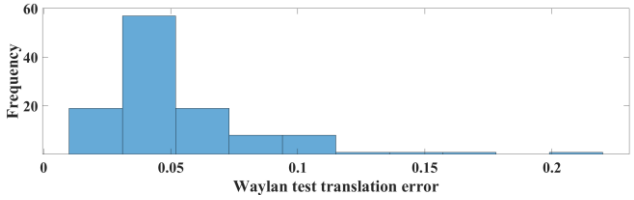

Figure 3. Distribution of the Wayland test translation error values for 115 wild-type mouse pupillometry datasets.

C. Recurrence Plot (RP) and Recurrence Quantification Analysis (RQA)

In this study, the calculated RP size was 10000x10000 points. The threshold, which is a parameter required for RP calculation, was chosen as 5% of the size of the trajectory reconstructed in the phase space. To obtain quantitative measures of the pupillometry dynamical characteristics, RQA [9] was conducted on obtained RPs. In this study, the following RQA measures were calculated: determinism (DET), maximal diagonal line length (L_{max}), average diagonal line length (L), and entropy (ENTR). Obtained results are summarized in Table 1.

TABLE I. SUMMARY OF THE RECURRENCE QUANTIFICATION ANALYSIS RESULTS

Stats	RQA measure			
	DET	***L***	***L_{max}***	***ENTR***
min	0.736	4.167	28	1.807
max	0.997	59.931	9499	3.801
mean	0.966	23.630	3036.435	2.937
deviation	0.040	11.982	2647.737	0.425

D. Largest Lyapunov Exponent (LLE)

The distribution of calculated for all data LLEs is shown in Fig. 4 (blue bars). As seen in Fig. 4, all the LLEs are positive, however, the presence of noise cannot be overlooked as a factor that can cause overestimation of the LLE value. To address the problem of noise in experimental data the method of surrogate data was applied to verify the results of the LLEs calculation. For each pupillometry time series 100 Fourier transform phase-randomized surrogates [11] were generated, and its LLEs were calculated. Then it was tested whether or not the original time series LLE is within its surrogates' LLEs distribution. Figure 4 demonstrates the distribution of 100 surrogate data LLEs (yellow bars with dashed line border) and the LLE value (red diamond-shaped mark) of the original time series (shown in Fig. 1) to which these surrogates correspond.

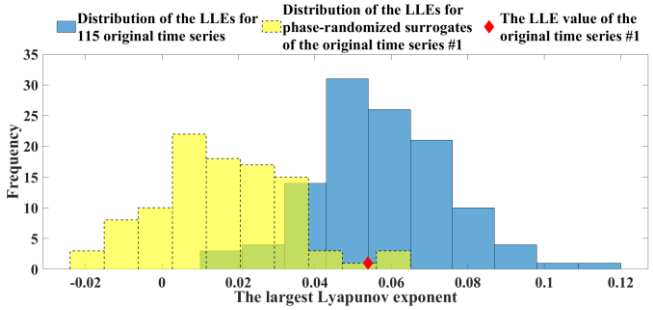

Figure 4. Results of the Lyapunov exponent estimation. Distribution of the largest Lyapunov exponents for 115 wild-type mouse pupillometry datasets (blue bars), the value of the largest Lyapunov exponent of the dataset number 1 (red diamond-shaped mark), and the distribution of the largest Lyapunov exponents calculated for the 100 phase-randomized surrogates corresponding to the dataset number 1 (yellow bars with dashed line outline).

E. Deterministic Nonlinear Prediction

Investigated data predictability was estimated by the deterministic nonlinear prediction method. The correlation coefficient (CC) between original and predicted data for the time series in Fig. 1 is shown in Fig. 5 (red dashed line), where the prediction step indicates how many data points ahead system state is predicted, and each step corresponds to the physical time equal to the sampling step. The distribution of the CC for the 10 steps prediction for all data is shown in Fig. 6. Similarly to the LLE results, the phase-randomized surrogate data were used to test noise influence on the prediction performance. The CC curves characterizing predictability for 50 surrogate time series are shown in Fig. 5 (black solid lines).

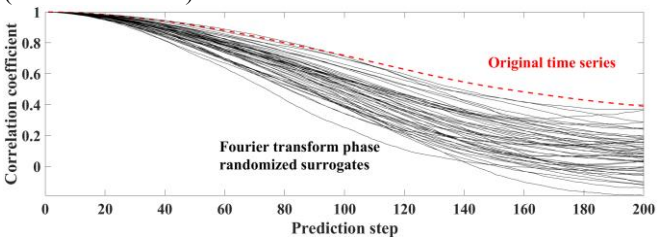

Figure 5. Deterministic nonlinear prediction results. The correlation coefficient between original and predicted data for the original pupillometry time series (red dash-dotted line) and for its 50 surrogates (black solid lines).

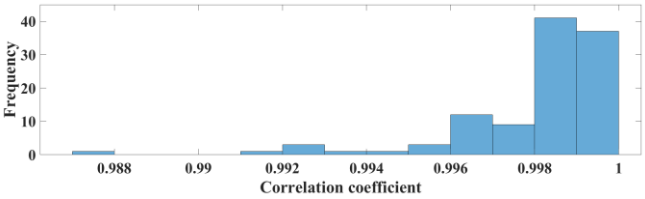

Figure 6. Distribution of the correlation coefficient for 10-steps forward prediction for 115 wild-type mouse pupillometry datasets.

IV. DISCUSSION

The conducted analysis aimed to test different properties of WT mouse spontaneous pupillary fluctuation dynamics [5], in order to clarify whether these properties are similar to those of healthy human subjects reported previously to be deterministic and chaotic [1,2]. First, the minimum embedding dimension – offering an estimate of the number of variables in the dynamical system involved in the creation of pupillometry dynamics – was estimated as 4, which might reflect, for example, the number of major neuromodulatory systems (ACh, NE, DA, 5HT) affecting process dynamics. The 4-dimensional time-delay-reconstructed trajectories calculated for all datasets demonstrated the presence of a certain structure which implies the existence of an attractor. Based on the obtained trajectories, methods of nonlinear time series analysis were applied to the collected data.

One of the primary goals of this study was to demonstrate that similar to human subjects, mouse pupillometry dynamics are deterministic. Pupillometry dynamics are extremely complex, possibly due to interaction with intricate fluctuations produced by the brain. At the same time, experimental data tend to contain a certain amount of noise of unknown nature, which could be almost indistinguishable from the process dynamics. Therefore, rather than relying on a single test, multiple methods are preferable to test the data's dynamical properties. We diagnosed the determinism of pupillometry dynamics using three methods: the Wayland test, RQA's DET calculation, and short-term predictability estimation. The WTE values close to 0 (Fig. 3), DET values close to 1 (Table 1), and presence of short-term predictability (Fig. 5 and 6)

identified strong underlying determinism in the data. However, as seen in Fig. 3 and Table 1, calculated values for several datasets were larger than 0.1 and less than 0.9 for WTE and DET, respectively. This means that a certain amount of noise is likely to be mixed in the data. The presence of observational noise is almost unavoidable for experimental data. At the same time, the biological system itself may produce a system or dynamic noise. Distinguishing types of noise and its levels mixed into the data is highly challenging, but an increase in the WTE value is expected to reflect an overall increase in the noise level [12,13]. Therefore, we can conclude that we are dealing with noise-contaminated deterministic data. Additionally, it is worth mentioning that while the presence of dynamical noise makes dynamics analysis more complicated, once extracted from the signal, dynamical noise may provide important information itself.

As the next step, the LLE was calculated to clarify the trajectories divergence rate. As seen from the distribution in Fig. 4, all LLEs were positive, however, their values were rather small. Positive LLE value is traditionally recognized as an indicator of deterministic chaos; however, data length limitations, imperfection of existing LLE estimation algorithms, as well as presence of noise in the data may lead to misestimation of the LLE. Trajectory divergence is also linked to the RQA's L_{max} measure, which is the inverse of the divergence. On average, large L_{max} (Table 1) indicates small divergence in the investigated data, which is consistent with the obtained LLE values. Several datasets demonstrated low L_{max} values. Taking into account that the same datasets have higher WTE and lower DET values, low L_{max} values are likely due to the effect of noise in the data, as the noise disturbance of the trajectory leads to misidentification of the neighborhood and the RP diagonal line interruption.

Taking into account the noise contamination issue, to verify the reliability of the obtained LLE results, the method of surrogate data was applied. When there is a clear separation between the results corresponding to the original time series and the surrogate data results, or if the original results lie within less than 5% of the surrogate results distribution, we can assume reliability of the obtained results despite the presence of noise. However, the comparison of the results of the LLE estimation with the distribution of corresponding surrogate data LLEs demonstrated that the original time series LLE was placed close to an inconclusive 5% of surrogate LLEs distribution. This indicates that there might be a significant influence of noise on the data in the LLE calculation results, thus leaving open the question whether the calculated positive LLEs can be treated as an indicator of deterministic chaos in the data.

Another calculated RQA measure is the average diagonal line length, L, which can be interpreted as the mean prediction time. As seen in Table 1, data predictability was recognized as relatively low, which is consistent with the results of the deterministic nonlinear prediction shown in Fig. 5. Additionally, high ENTR values seen in Table 1 indicated the high complexity of the system, as well as its low predictability [9, 14]. Taking into account the results of the surrogate data method application, a fast decrease in prediction quality with time is likely to be caused by the presence of noise in the experimental data.

V. Conclusion

This study aimed to investigate properties of WT mouse spontaneous pupillary fluctuation dynamics by the methods of nonlinear time series analysis. Results clearly indicated strong underlying determinism in the data, which, however, was altered in several datasets by a considerable amount of noise. As pupillometry dynamics of human subjects is recognized to be deterministic [1,2], we can assume determinism of spontaneous pupillary fluctuations regardless of species. The presence of determinism is essential for further applied studies on pupillary data.

Additionally, predictability and trajectory divergence rates were also investigated. Although the obtained characteristics were similar to those of time series produced by chaotic dynamics, results of surrogate data method application indicated that the effect of noise in the data cannot be overlooked, and further careful investigation of WT mouse pupillometry data is required.

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