First evidence that intrinsic fetal heart rate variability exists and is affected by chronic hypoxia

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Abstract

INTRODUCTION: It is established that normal fetal HRV represents the integration of the autonomic nervous system, components due to fetal body and breathing movements, baroreflex and circadian processes. There is also some evidence of possible intrinsic pacemaker rhythms of the SA node affecting HRV in adult disease. However, whether intrinsic HRV (iHRV) exists in the fetal period and whether this is affected by chronic fetal hypoxia has never been tested. Here, we show iHRV in isolated hearts from fetal sheep in late gestation and significant effects on iHRV of pregnancy complicated by chronic fetal hypoxia.

METHODS: Chronically catheterized ewes carrying male singleton fetuses were exposed to normoxia (n=6) or hypoxia (10% inspired O2, n=9) for the last third of gestation (105-138 dG; term~145 dG) in bespoke isobaric chambers, as before. At 138dG, isolated hearts were studied under a Langendorff preparation. CIMVA software was used to calculate basal iHRV matrix indices across five signal-analytical domains from the systolic peaks within 15 min segments in each heart, as before. Data presented as Mean+SEM were compared by the Student's t test for unpaired data.

RESULTS: This level of maternal H yields fetal PaO2 values of 11.5±0.6 relative to controls of 20.9±0.5 mmHg. Hearts isolated from H pregnancy showed approximately 4-fold increases in the Grid transformation feature as well as the AND similarity index (sgridAND, informational domain) and a 4-fold reduction in the Scale dependent Lyapunov exponent slope (SDLEalpha, invariant domain). We also detected a 2-fold reduction in the Recurrence quantification analysis, the percentage of laminarity and recurrences and maximum diagonal line (pL, pR, dlmax, all from geometric domain) and (AsymI, energetic domain). For dlmax and sgridAND measures, this is also correlated to left ventricular end-diastolic pressure (LVEDP) across both groups.

CONCLUSIONS: The isolated fetal hearts from the hypoxic pregnancy group exhibit a lower complexity in iHRV. This is the first evidence that iHRV originates in fetal life and that chronic fetal hypoxia significantly alters it.

Significance Statement

Fetal heart rate variability (fHRV) is an important indicator of health and disease, yet its physiological origins are poorly understood. Both, the heart, intrinsically, and extrinsic systems, such as the brain, are hypothesized to contribute to HRV. In a near-term fetal sheep model of human development, we identified fHRV components reflecting intrinsic contributions to fHRV. In addition, we show that these intrinsic fHRV components carry memory of chronic oxygen deprivation the fetus experienced during the last third of gestation.

Introduction

Antenatal electronic monitoring of fetal heart rate (FHR) is widely used clinically and is an important tool to assess fetal condition. FHR monitoring is routinely used to assess fetal wellbeing in pregnancies affected by utero-placental dysfunction and profound, prolonged alterations in FHR variability (fHRV) are thought to represent acute fetal compromise.(1)

Persistent reductions in short term variation (STV), a commonly used obstetrical indicator of fHRV, below 3 ms in the antenatal period, within 24 hours of delivery, is moderately predictive of an increased risk of metabolic acidosis in the neonate at birth, and early neonatal death.(2)(3) As such, STV, as part of computerised cardiotocography (cCTG) examination, is not recommended as a sole means of antenatal surveillance of human fetuses with suspected utero-placental dysfunction and chronic fetal hypoxia to detect acute fetal distress.(4) Further studies on the usefulness of fHRV for prediction of fetal hypoxia and acidemia are needed and should involve other fHRV measures and modalities of recording.(5) While the empirical search for adequate measures of fHRV that predict fetal distress continues, the physiological basis underlying the impact of hypoxia and acidemia on FHRV remains unclear.

It is established that normal FHR variability (fHRV) represents a complex, nonlinear integration of the sympathetic and parasympathetic nervous system activities. Fetal body and breathing movements, sleep states (6) as well as baroreflex and circadian processes also influence fHRV.(7–10) There is some evidence that intrinsic pacemaker rhythms of the sino-atrial node affect HRV in critically ill adults.(11) However, whether intrinsic HRV (iHRV) exists in the fetal period and whether this is affected by chronic fetal hypoxia has never been tested.

The isolated Langendorff *ex vivo* preparation of fetal sheep heart is perfectly suited for measuring iHRV, because it is devoid of any extracardial innervation or systemic hormonal influences thus representing a model of studying iHRV in its “pure autochthonic form”.

Here, we show iHRV in isolated hearts from fetal sheep in late gestation. We also show that chronic fetal hypoxia has significant effects on iHRV.

Results

Maternal P_aO_2 was reduced in hypoxic pregnancy (42.0±1.2mmHg vs. 105.7±3.7 mmHg, P<0.05). Fetuses exposed chronic hypoxia had a significant reduction in the partial pressure of arterial oxygen (11.5±0.6 mmHg vs. 20.9±0.5mmHg, P<0.05).

Hearts isolated from chronically hypoxic fetuses showed distinct changes in iHRV measures across four signal-analytical domains (Fig. 1). There were approximately 4-fold increases in the Grid transformation feature as well as the AND similarity index (sgridAND, informational domain) and a 4-fold reduction in the Scale dependent Lyapunov exponent slope (SDLEalpha, invariant domain). We also detected a 2-fold reduction in the Recurrence quantification analysis, the percentage of laminarity and recurrences and maximum diagonal line (pL, pR, dlmax, all from geometric domain) and (AsymI, energetic domain). There was a moderate fall in the Detrended fluctuation analysis (area under the curve, DFA AUC, invariant domain). In summary, the isolated fetal hearts from the hypoxic pregnancy group exhibit a lower complexity in iHRV. For dlmax and sgridAND measures, this is also correlated to left ventricular end-diastolic pressure (LVEDP) across both groups (Fig. 2 and 3).

Discussion

Our findings imply that fHRV monitoring can reveal intrinsic cardiac function. FHRV has served as a scientific and diagnostic tool to quantify the fluctuations of cardiac activity under various conditions since the early 1980's.(12) However, little is known about its biological origins. From studies of healthy adult subjects during exercise and studies of heart-transplant recipients, we know that intrinsic components of cardiac rhythm contribute substantially to HRV.(13–15)

Our findings provide the first evidence that iHRV originates in fetal life and that chronic fetal hypoxia significantly alters it. The relationship between nonlinear measures of fHRV and LVEDP suggests that such fHRV measures may reflect fetal myocardial function. Therefore, they may be clinically useful as a biomarker of cardiac reserve and decompensation during antepartum or intrapartum monitoring. Future studies should examine the potential of such fHRV measures to identify fetuses experiencing chronic hypoxia and other conditions, such as infection, that may impact myocardial function before and after birth.

The findings raise several exciting questions. What is the transfer mechanism by which *in utero* hypoxia imprints upon iHRV? May it be via the impact on myocardiogenesis, which then impacts patterns of contractility that we measure by LVEDP? Does the putative transfer mechanism of i*n utero* hypoxia upon iHRV features depend upon vagal and sympathetic fluctuations *in vivo* or is it entirely autochthonic, emerging from the adaptive processes within the excitatory cells in response to chronic hypoxia?

Findings in fetal sheep subjected to a labour-like insult with worsening acidemia and the work in adult animal models of acidemia indicate that around a pH of 7.2, the physiological myocardial activity is curbed via the Bezold-Jarisch-like reflex. This is a vagally mediated myocardial depression reflex that reduces cardiac output under conditions of moderate acidemia; it is thought to preserve depleting myocardial energy reserves.(16–20)

When labour is associated with worsening acidemia, cardiac decompensation contributes to brain injury.(21) Acidemia impacts myocardial contractility, which decreases cardiac output and arterial blood pressure. It is not yet understood how hypoxic-acidemia may disrupt sinus node pacemaker activity, thus disrupting iHRV.

What may the identified iHRV features dlmax and sgridAND, which correlate to LVEDP, represent physiologically? The first feature is derived from a recurrence plot where the diagonal lines represent the trajectory visiting the same region of the phase space at different times. The lengths of diagonal lines in a recurrence plot are related to the predictability of the system dynamics. Perfectly predictable systems would have infinitely long diagonal lines in the recurrence plot (high dlmax). Stochastic and chaotic systems would have very short diagonal lines (low dlmax).(22–24) In our case, hypoxia reduces dlmax of iHRV, which correlates to an increased LVEDP compared to normoxic fetuses.

The grid transformation AND similarity index (sgridAND) measures the dynamical system phase space reconstruction trajectory, with a specific embedding dimension and time delay. It is binarized over a grid (*i.e.*, pixel visited by the trajectory=1, all others=0) to produce an image. Two grid images corresponding to different time delays or different windows in time are then compared. (25) The sgridAND measure is the normalized sum of the binary AND operation on the two compared images and represents a similarity index between the phase space trajectory from two consecutive windows. Low values indicate that the iHRV dynamics have changed while high values mean the dynamics are similar. The latter is the case for hypoxic fetuses' iHRV and correlates again with higher LVEDP.

Together, our findings indicate that *in utero* hypoxia reduces the short-term predictability of iHRV and increases its long-range similarity (note the phase space structure in the example of the hypoxic iHRV shown in Fig. 3). Both effects do not contradict each other, because the effects are captured in different signal-analytical domains, one being a geometric feature of iHRV and another referring to longer-term temporal processes in the informational domain. Importantly, both changes result in a consistent increase of LVEDP, a rare demonstration that a complex HRV property is linked to a clinical correlate in a meaningful way.

In future work we will test whether the mechanism by which memory of *in utero* chronic hypoxia is imprinted into iHRV and affects the LVEDP, is via alterations in the efficiency of sinu-atrial signal transduction that alter synchronization properties within the sinus node pacemaker cells.

Ultimately, our approach should lead to a battery of iHRV biomarkers that can be captured non-invasively from fetal ECG. This may contribute to a more timely identification of cardiovascular

decompensation, such that selective postnatal treatment with erythropoietin or hypothermia may be deployed to prevent incipient brain injury. (1, 5, 16)

Methods

Surgical Preparation

All experiments were performed in accordance with the UK Home Office guidance under the Animals (Scientific Procedures) Act 1986 and were approved by the Ethical Review Committee of the University of Cambridge.

Briefly, chronically catheterized ewes carrying male singleton fetuses were exposed to normoxia (n=6) or hypoxia (10% inspired O2, n=9) for the last third of gestation (105-138 dG; term~145 dG) in bespoke isobaric chambers, as before (Fig. 4).(26) At 138dG, isolated hearts were studied under a Langendorff preparation.

At 100±1 days gestational age (term ca. 145 days), pregnant Welsh mountain ewes carrying singleton pregnancies determined by ultrasound scan (Toshiba Medical Systems Europe, Zoetermeer, the Netherlands) underwent a laparotomy under general anesthesia. In brief, food but not water was withdrawn for 24 h prior to surgery. Anesthesia was induced by Alfaxan (1.5–2.5 mg kg1 i.v. alfaxalone; Jurox Ltd., Worcestershire, UK) and general anesthesia (1.5–2.0% isoflurane in 60:40 O2:N2O) maintained by use of a positive pressure ventilator (Datex-Ohmeda Ltd., Hatfield, Hertfordshire, UK). Antibiotics (30 mg kg1 i.m. procaine benzylpenicillin; Depocillin; Intervet UK Ltd., Milton Keynes, UK) and an analgesic (1.4 mg kg1 s.c. carprofen; Rimadyl; Pfizer Ltd., Kent, UK) were administered immediately before the start of surgery. Following a midline abdominal incision and uterotomy, the fetal hind limbs were exposed and the fetal sex was determined. If male, then the fetuses were chosen for this study. Female fetuses were used for another experiment. The fetus was returned into the intrauterine cavity, and the uterine and maternal abdominal incisions were closed in layers. A Teflon catheter (i.d. 1.0 mm, o.d. 1.6 mm, Altec, UK) was then placed in the maternal femoral artery and extended to the descending aorta, in addition to a venous catheter extended into the maternal inferior vena cava (i.d. 0.86 mm, o.d. 1.52 mm, Critchly Electrical Products, NSW, Australia). Catheters were filled with heparinized saline (80 I.U mL1 heparin in 0.9% NaCl), tunneled subcutaneously, and exteriorized via a keyhole incision made in the maternal flank to be kept inside a plastic pouch sewn onto the maternal skin. Inhalation

anesthesia was withdrawn and the ewe was ventilated until respiratory movements were observed. The ewe was extubated when spontaneous breathing returned and moved into a recovery pen adjacent to other sheep with free access to food and water. A total of 18 Welsh Mountain ewes carrying male singleton fetuses were surgically instrumented for this study.

Postoperative care

Following surgery, ewes were housed in individual floor pens with a 12 h:12 h light:dark cycle and free access to hay and water. Antibiotics (30 mg kg1 i.m. procaine benzylpenicillin; Depocillin; Intervet UK Ltd., Milton Keynes, UK) were administered daily to the ewe for 5 days following surgery. From 103 days of gestation, ewes were fed daily a bespoke maintenance diet made up of concentrate and hay pellets to facilitate the monitoring of food intake (Cambridge ewe diet: 40 g nuts kg1 and 3 g hay kg1 ; Manor Farm Feeds Ltd.; Oakham, Leicestershire, UK). Generally, normal feeding patterns were restored within 24–48 h of recovery. On day 103 of gestation, ewes were randomly assigned to either of two experimental groups: normoxia (N: n = 6) or chronic hypoxia (H: n = 9).

Ewes allocated to chronic hypoxic pregnancy were housed in one of four bespoke isobaric hypoxic chambers (Telstar Ace, Dewsbury, West Yorkshire, UK; Fig. 1). These chambers were supplied with variable amounts of nitrogen and air provided via nitrogen generators and air compressors, respectively, from a custom-designed nitrogen-generating system (Domnick Hunter Gas Generation, Gateshead, Tyne & Wear, UK). The system operated continuously, automatically switching between adsorption beds of two nitrogen generators (Domnick Hunter N2MAX112 9 2) to ensure a constant provision of pure nitrogen gas. The purity of the nitrogen was monitored to ensure only gas of the required purity reached the application. Compressed air and compressed nitrogen were then piped to the laboratory containing the hypoxic chambers and gases were blended to requirements. The inspirate air mixture underwent a minimum of 12 changes per hour in each chamber and the incoming air mixture was passed via silencers able to reduce noise levels within the hypoxic chamber laboratory (76 dB(A)) and inside each chamber (63 dB(A)) to values lower than those necessary to

abide by the Control of Noise at Work Regulations. This not only complied with human health and safety and animal welfare regulations, but also provided a tranquil environment for the animal inside each chamber. All chambers were equipped with an electronic automatic humidity cool steam injection system (1100- 03239 HS-SINF Masalles, Barcelona, Spain) to ensure appropriate humidity in the inspirate (55 10%). Ambient PO2, PCO2, humidity, and temperature within each chamber were monitored via sensors, displayed, and values recorded continuously via the Trends Building Management System of the University of Cambridge through a secure Redcare intranet. In this way, the percentage of oxygen in the isolators could be controlled with precision continuously over long periods of time. For experimental procedures, each chamber had a double transfer port to internalize material and a manually operated sliding panel to encourage the ewe into a position where daily sampling of blood could be achieved through glove compartments (Fig. 1). Each chamber incorporated a drinking bowl on continuous water supply and a rotating food compartment which could be removed for determining food intake. The chambers were transparent, allowing ewes to visualize each other. A transfer isolation cart could couple two chambers together, permitting ewes to move transiently to an adjacent chamber maintained at the same oxygen environment. This was necessary for cleaning the chambers, which occurred once per week. Therefore, all experimental and maintenance procedures could be carried out without interruption of the hypoxic exposure. Pregnancies assigned to the chronic hypoxia group were placed inside the chambers at 103 days of gestation under normoxic conditions (11 L sec1 air, equating to 39.6 m3 h1). At 105 days, pregnancies were exposed to approximately 10% O2 by altering the incoming inspirate mixture to 5 L sec1 air: 6 L sec1 N2.

Langendorff preparation

Fetal hearts were removed, rapidly connected to a langendorff apparatus and perfused at a constant pressure of 30 mmHg, as detailed by Fletcher et al (2005).(27) The ductus arteriosus was ligated. Pulmonary arteriotomy was performed. A recirculating solution of Krebs-Henseleit bicarbonate buffer containing ($mM.L^{-1}$) 120 NaCl, 4.7 KCl, 1.2 $MgSO_2.7H_2O$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 glucose, and 1.3 $CaCl_2.2H_2O$ was filtered through a 5 µm cellulose nitrate filter (Millipore, Bedford, MA, USA) and gassed with O_2:CO_2 (95:5) at 37°C. A

small flexible non-elastic balloon was inserted into the left ventricle through the left atrium. The balloon was filled with deionised water and attached to a rigid deionised water-filled catheter connected to a calibrated pressure transducer (Argon Medical Devices, Texas, USA). The balloon volume was adjusted to obtain a left ventricular end diastolic pressure (LVEDP) recording of approximately 5-10 mmHg. After an initial 15 min stabilisation period, basal heart rate (HR), left ventricular systolic pressure (LVSP) and LVEDP were recorded. Basal left ventricular developed pressure (LVDP) was calculated as LVSP-LVEDP. The maximum and minimum first derivatives of the left ventricular pressure (dP/dt_{max} and dP/dt_{min}) were calculated using an M-PAQ data acquisition system (Maastricht Programmable AcQuisition System, Netherlands).

For HRV analysis purpose, all original recording traces of left ventricular pressure were exported to LabChart® 7 software (ADInstruments, UK).

FHRV analysis

To derive fHRV fetal left ventricular pressure recordings sampled at 1kHz were analyzed with CIMVA (continuous individualized multiorgan variability analysis) software, as before.(28) Interbeat intervals were extracted from the pressure recordings using the systolic peaks. A range of 55 basal iHRV indices was then calculated across five signal-analytical domains from the interbeat interval time series, within 15 min segments in each heart, determined as an average of three non-overlapping 5 min intervals.

Statistical analysis

Student T test for unpaired data was used to compare variables from the hypoxic versus the normoxic group assuming significance for $P<0.05$ (SigmaStat). Results are presented as Mean$\pm$SEM.

Acknowledgements

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Figure legends

Figure 1. Effects of chronic hypoxia during pregnancy on fetal intrinsic heart rate variability (iHRV). Values are mean ± SEM. Groups are normoxic (N, n=6) and hypoxic (H, n=9). Significant differences are: * vs. N, $P<0.05$ (Student's t test for unpaired data).

Figure 2. Correlation of left ventricular end-diastolic pressure (LVEDP) and the intrinsic heart rate variability (iHRV) measures dlmax and sgridAND. These measures are shown because, among the iHRV measures shown in Fig. 1, these two measures were identified as correlating to LVEDP in normoxic (empty circles) and hypoxic (black circles) groups.

Figure 3. A representative beat-to-beat time series, followed by the corresponding grid transformation AND similarity index (sgridAND) and the recurrence plots are shown for a normoxic (right) and hypoxic (left) fetus to demonstrate iHRV pattern differences revealed with such representation of the phase space organization of the beat-to-beat fluctuations.

Figure 4. LEFT: Experimental protocol for *ex vivo* analyses. **MIDDLE**: Isobaric hypoxic chambers and nitrogen-generating system.(44) **RIGHT**: Isolated heart perfusion model.(46)

Figure 1.

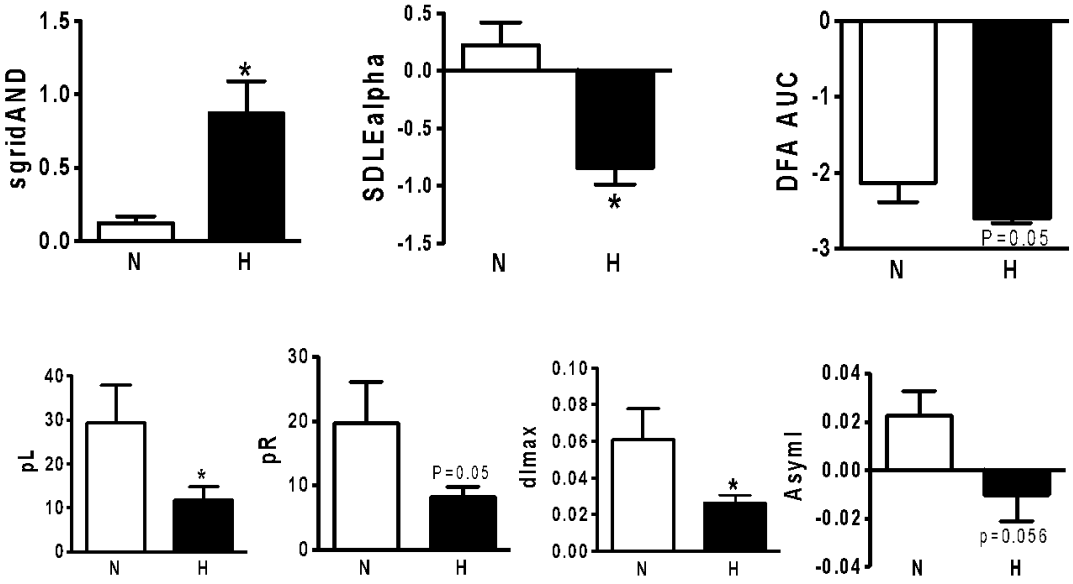

Figure 2.

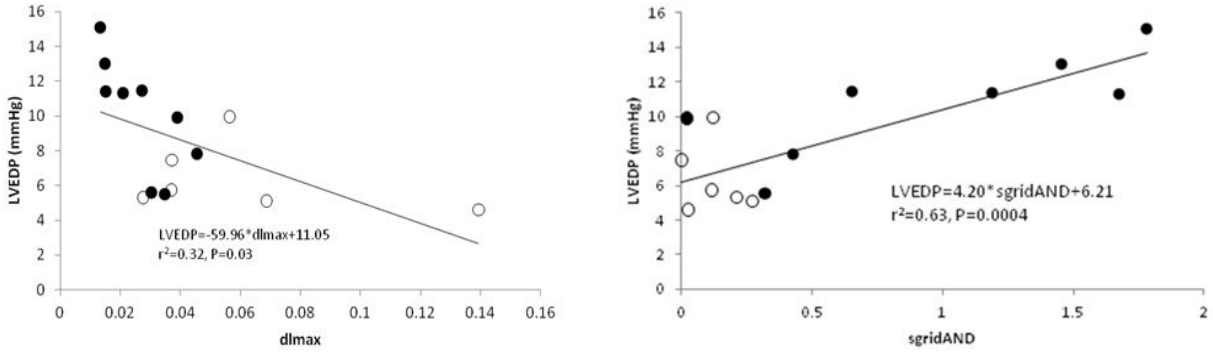

Figure 3.

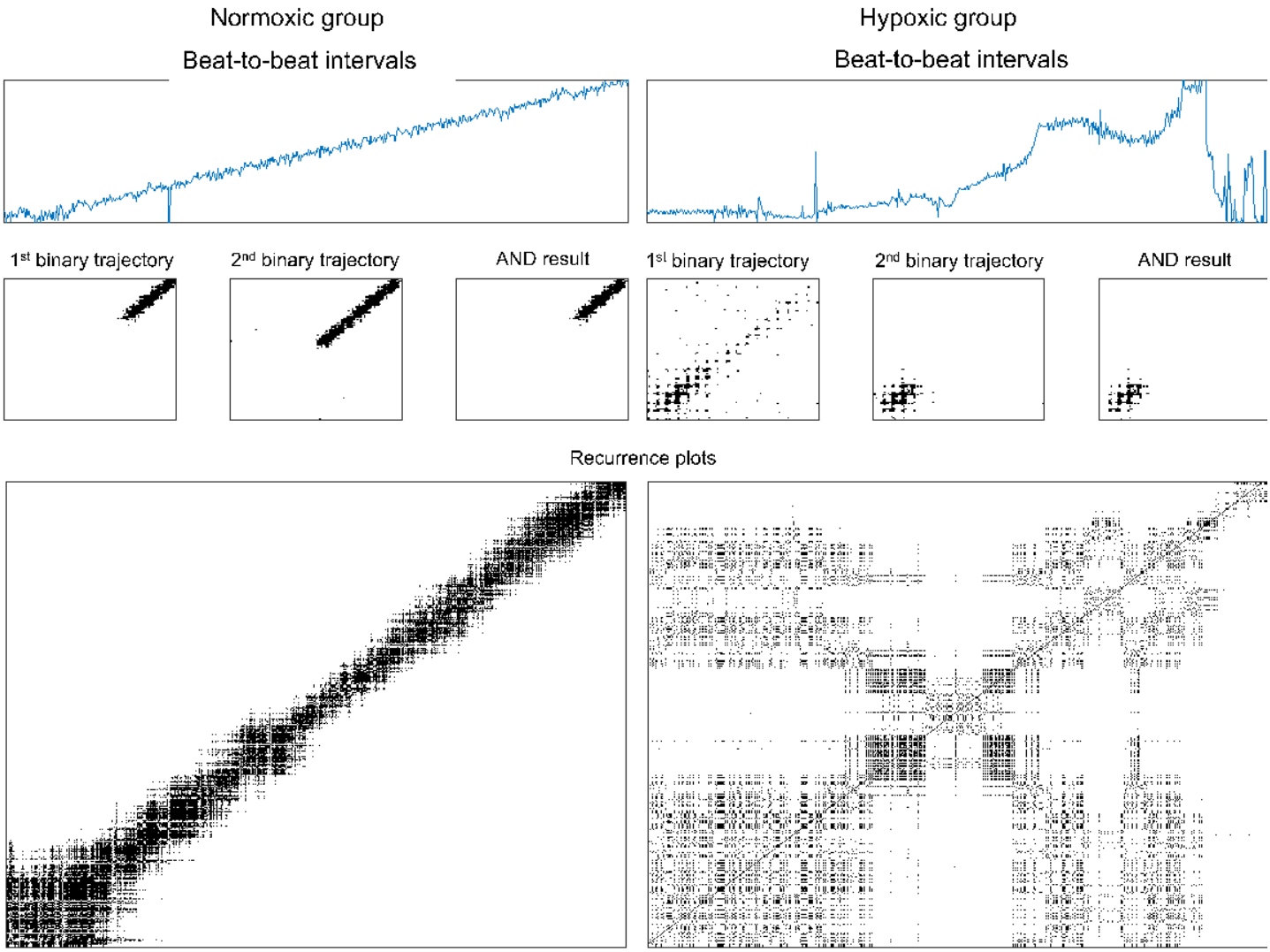

Figure 4.