Fetal development of complex autonomic control evaluated from multiscale heart rate patterns

Dirk Hoyer,1 Samuel Nowack,1,* Stephan Bauer,1* Florian Tetschke,1* Anja Rudolph,1,2* Ulrike Wallwitz,1,2* Franziska Jaenicke,1,2* Esther Heinicke,1,2* Theresa Götz,1 Ralph Huonker,1 Otto W. Witte,1 Ekkehard Schleussner,2 and Uwe Schneider2

1Jena University Hospital, Biomagnetic Center, Hans Berger Department of Neurology, Germany; 2Jena University Hospital, Department of Obstetrics, Germany

Submitted 27 March 2012; accepted in final form 14 December 2012

There is increasing consensus that a certain number of diseases such as cardiovascular disease, metabolic syndrome, atherosclerosis, Type 2 diabetes, learning difficulties, hyperkinetic disorders, as well as cognitive, behavioral, and emotional problems in late postnatal age are associated with adverse influences during fetal development that became permanently programmed (1, 2, 4, 10, 11, 19, 20, 23, 24, 26, 31, 32, 39, 40, 44, 47). However, early identification of fetal developmental problems is a challenging topic of prenatal diagnosis due to the extremely limited number of observable variables from the fetus. During the fetal maturation the emerging complex behavioral patterns are associated with an increasing functional integration in the organism. In that connection the autonomic nervous system (ANS) plays a predominant role for complex coordinated control of multiple vitally important physiological subsystems in the organism. Since ANS activity is reflected in the heart rate pattern, an appropriate analysis of heart rate variability (HRV) may provide essential information regarding the individual fetal development.

For prenatal diagnosis, it is helpful to understand how indices of complex system behavior characterize fetal development. Hence, it is useful to determine whether the fetal developmental stage can be classified by these indices and whether the formation of this complex system behavior is governed by specific rules that can improve our understanding of the fetal maturation process. The latter leads to the suggestion that consideration of the universal rules of nonlinear dynamics and self-organization in nature and developmental biology (3, 9, 21, 28, 37) may be beneficial for assessing fetal growth and maturation based on HRV indices.

Magnetocardiographic recordings allow a noninvasive assessment of fetal heart rate patterns on a high temporal beat-to-beat resolution according to ANS-mediated rhythms. Established HRV characteristics traditionally focused on the contribution of the different branches of the ANS (6, 7, 14, 41, 42, 50–52) but did not directly address the system’s theoretic rules of development. On the other hand, clinically established criteria of fetal maturation, such as the increasing number and extent of movement-related heart rate accelerations and the formation of active and quiet states with increasing duration and differentiation (33), are consistent with general developmental characteristics and indicate the potential of a correspondingly focused HRV analysis. From single-scale HRV measures contradictory results are reported, such as increasing complexity during fetal growth and maturation as well as decreasing complexity in connection with the formation of heart rate accelerations patterns (14, 49, 52).

Traditional HRV analysis is based on natural rhythms such as the heartbeat period itself, the high-frequency band assigned to vagal heart rate modulations, the low-frequency band assigned to vagal and sympathetic heart rate modulations, and the very-low-frequency band (VLF) integrating several autonomic and humoral control loops. The limitation of this simplification already becomes obvious when one takes into account that sympathovagal interactions operate both in an antagonistic and
synergistic manner and show that HRV cannot selectively assess sympathetic cardiac activity. Moreover, it is self-evident that the isolated activity of one particular autonomic branch cannot explain the complex integrative control mediated by the ANS. Hence, it is not surprising that integrative indices of conventional linear analysis [standard deviations of NN intervals (SDNN), VLF, see Table 1] and potentially more adequate complexity indices provided relevant diagnostic and prognostic values in several clinical studies of adult patients (e.g., 13, 18, 25, 36, 43) as well as with regard to the normal fetal maturation (e.g., 16, 48, 52).

Universal aspects of the behavior of growing and developing complex systems incorporate an increasing number of functional elements while forming integrated behavior patterns (3, 9, 21, 28, 37). A system order parameter, such as heart rate fluctuation, should reflect these aspects. An increasing number of integrated fluctuating elements should increase the fluctuation amplitude (SDNN). However, the emerging heart rate patterns are fundamentally not normally distributed. The asymmetry of sympathovagal innervation with their different time constants can be expected to be reflected in the skewness of the heartbeat interval distribution. Traditional power spectral analysis considers the signal power over the scale of frequencies. Power widely spread over all frequencies indicates high irregularity in contrast to distinct peaks that indicate predominant rhythms and, hence, regular patterns. The physiological interpretation of multiscale complexity is less clear since regulating influences in a complex dynamic system can affect all scales (5, 12, 15, 17). Scales of low complexity indicate regular patterns with respective periodicity. But isolated scales of low complexity that would indicate the periodicity of one single frequency oscillation typically do not appear in complex systems. Accordingly, in complexity analysis, appearance of ranges of correlated neighboring scales is typical (5, 16).

In preceding studies that were independent of the fetal state, we found a shift from short heart rate decelerations to increasing longer lasting heart rate accelerations in connection with increasing gestational age (14). The formation of these movement-related heart rate accelerations indicates an increasingly coordinated and hence integrated behavior. In a multiscale entropy (MSE) analysis of age subgroups (20–29 WGA vs. 35–40 WGA), we found increasing short scale and decreasing long scale complexity (16). In any growing and developing system, a rise in complexity is a typical sign of the increasing number of elements involved, whereas decreasing complexity is seen as an indication of synchronization and regularization of these elements. In a simple linear analysis, emerging integrative ANS function was demonstrated by autocorrelations that spread out over increasing time scales in association with increasing gestational age (53). However, complexity was found to be maturation related, increasing as well as decreasing, and has been discussed in connection with the formation of behavioral states (8, 14, 16, 49); however, a systematic analysis of a representative data set of classified states is lacking.

The objective of the present work was to explore fetal developmental indices related to functional integration of autonomic control in predominant behavioral states. With respect to the developing respective heart rate patterns, we investigated the standard deviation of heartbeat intervals (SDNN), skewness, dominance of frequency bands, and multiscale entropy during active and quiet states.

### MATERIALS AND METHODS

**Investigation flow.** The following steps were used or made for this protocol: 1) MCG measurement of 294 subjects over 30 min; 2) fetal heartbeat detection, calculation of the normal-to-normal (NN) heartbeat interval series; 3) selection of active and quiet state intervals over 10 min and constitution of two subsets for separate analysis in each state, extension by a 30-min active state subset; 4) calculation of SDNN, skewness, power spectra density (PSD), multiscale entropy (MSE); and 5) analysis of the dependence of the calculated parameters on the fetal age for each state separately.

**Subjects.** The study database of the Biomagnetic Center/Department of Obstetrics consisted of 294 normal singleton fetuses, healthy according to standard obstetric observation methods single recording in a nonstress situation. The exclusion criteria were 1) maternal, administration of cardiovascular effective drugs, cardiovascular diseases, or diabetes; and 2) fetal, intrauterine growth restriction, non-reassuring nonstress test based on conventional CTG, known chromosomal abnormalities, or congenital abnormalities based on ultrasound diagnosis, fetal arrhythmia, and previous exposure to synthetic steroids in utero. The study was approved by the Local Ethics

<table>
<thead>
<tr>
<th>Table 1. Investigated HRV parameters</th>
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<td><strong>Parameter</strong></td>
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All frequency bands are chosen according to Ref. 6. Please notice that the gap between high frequency (HF) and low frequency (LF) reflects a frequency range without significant heart rate rhythms (6). HRV, heart rate variability; gMSE, generalized multiscale entropy.
Committee of the Friedrich Schiller University. All women signed a written, informed consent form.

Data acquisition. All measurements were taken in a magnetically shielded room at the Biomagnetic Center of the Jena University Hospital using the vector-magnetograph ARGOS 200 (ATB Chieti). Heartbeats were detected using a preprocessing toolbox of the own team (27) or one by BMDSys Jena.

The pregnant women were positioned supine or with a slight twist to either side to prevent compression of the inferior vena cava by the pregnant uterus. The dewar was positioned as close as possible above the fetal heart, determined by sonographic localization, but without contact to the maternal abdominal wall.

The magnetography (MCG) signal was recorded over a period of 30 min with a sampling rate of 1,024 Hz. The fetal heartbeats were automatically detected in those magnetic channels with the highest signal-to-noise ratio and subsequently confirmed by an expert analyst. In all analyzed data sets, we observed less than 2% incorrect detections and ectopic beats that were linearly interpolated to constitute artifact free data sets of normal-to-normal (NN) intervals of 30 min duration.

Assessment of fetal activity states. With reference to previous investigations, we distinguished between two qualities of fetal activity. These qualities were classified according to typical heart rate patterns (HRP) and will henceforth be referred to as “states.” In the studied data sets, subsets of 10 min length showing coherence with the characteristic heart rate pattern were selected after a consensus decision by three independent obstetricians according to the following standard criteria (29, 42). First, there is HRP I (quiet state, correlated to quiet sleep 1F) [stable fetal heart rate (fHR) (variation of visually determined floating baseline (< ±5 bpm from floating baseline fHR), isolated (maximum 2 per 10 min) accelerations (>15 bpm over >15 s), and a floating baseline fHR that does not exceed 160 bpm]. Second, there is HRP II [active state, correlated to active sleep 2F (fluctuating fHR with an oscillation bandwidth >±5 bpm from floating baseline, frequent (at least 3 per 10 min) accelerations (>15 bpm, >15 s), and the fHR exceeding 160 bpm only during accelerations].

HRP with long-lasting accelerations exceeding 160 bpm, frequently fused into a sustained tachycardia (correlated to active awake 4F), were not analyzed.

From the overall 294 recordings, 65 recordings included both an active and a quiet period, while 49 recordings contained only a 10-min active and 46 recordings contained only a 10-min quiet state classified period. Since the analysis was done separately for each state, all state-selected data sets were incorporated. The resulting study data set consisted of n = 248 active and n = 111 quiet state intervals. This relationship corresponds to the overall appearance of states (33).

Additionally, we included the remaining data subset of 30 min active states (n = 134) from the same data base.

Multiscale entropy. Scale analysis denotes the time resolution of the NN interval dynamics. The shortest time scale constitutes the original NN interval series. Larger scales are considered by accumulating neighboring NN intervals leading to lower time resolutions (low-pass filter) and increasing investigation time horizons by means of a coarse graining procedure (5, 45). From the resulting scale-dependent NN data, calculated entropy is organized as multiscale entropy (MSE). To validate the MSE analysis from 10-min data intervals, generalized MSE (gMSE) and refined MSE (rMSE) methodologies were introduced and their results were compared. For details see Appendix. In the main body, the results of using Butterworth low-pass filtering and resampling for scaling (45) and generalized mutual information for complexity estimation (33, 34) are presented.

Parameters. The investigated HRV parameters were selected according to universal characteristics of order parameters of growing and developing systems such as introduced above (see Table 1).

Statistical analysis. The MSE functions were plotted as mean and 95% confidence interval. The predictive value of the MSE parameters was evaluated by linear regression models over the entire maturation period \{coefficient of determination $R^2$, regression coefficient \{slope of regression line (95% confidence interval)\}, standardized regression coefficient $\beta\; P\; value\}. The quiet state ($n = 111$) and the active state ($n = 248$) were analyzed independent of each other.

$P < 0.05$ was considered significant. The most conservative Bonferroni correction according to the number of scales (factor 20) leading to $P < 0.001$ can be considered as a very safe $\alpha$ since complexity values were found to significantly correlate (Pearson’s correlation coefficient, two-sided) with their neighbors over at least five scales in all ranges. All statistical analyses were carried out using IBM SPSS Statistics 19.

RESULTS

Figure 1 displays typical fetal heart rate patterns during early and late gestational age windows characterizing quiet and active states, respectively. During the quiet state, the fluctuating baseline in the early gestational age (GA) recording changes toward a more constant baseline in the older fetus. In contrast, during the active state, fluctuations with higher amplitudes appear in the early GA recording in a rather nonsystematic form and develop toward clearly pronounced peaks of heart rate acceleration patterns over at least 15 s exceeding 15 bpm amplitude above baseline according to active state criteria (33). These heart rate characteristics demonstrate an age-dependent state differentiation toward 1 patterns with low amplitude oscillations indicating the formation of regular breathing-related heart rate modulations during the quiet state leading to the behavior state “quiet sleep” (Fig. 1, above), or 2) higher amplitude heart rate acceleration peaks indicating the formation of movement-related cardiovascular activation during the active state leading to the behavior state “active sleep” (Fig. 1, below) (33).

Histogram-based maturation parameters. SDNN significantly increased in both states; however, with a small coefficient of determination ($R^2 = 5.5\%$ and 6.6%). In contrast, with larger $R^2$, the regression line of skewness explained the variance of 20.4% in the quiet and 27.9% in the active state (Fig. 2, Table 2).

Multiscale Complexity

The MSE characteristics typically show a complexity drop from scale 1 to scale 2 and a complexity increase over the following scales 2–20, but not reaching the complexity level of noncorrelated Gaussian white noise (Fig. 3).

The example data subsets in Fig. 3 illustrate higher complexity in the quiet state compared with the active state together with a stronger state difference in the older subgroup compared with the younger subgroup. The latter result is consistent with the well-known maturation-related formation of different activity states and the respective experiences of the state classifying obstetricians.

The following analysis focuses on maturation using the complete age data set over all recordings for each preclassified state separately.

Multiscale entropy-based maturation parameters. The relationships between scale-wise complexity and gestational age show typical patterns that are similar in both states (see Fig. 4, Table 2). However, in the active state, the age dependencies are clearly lower, and pattern seems to shift toward larger scales.
During the quiet state, the strongest age-related complexity increase was found around scale 3, while remaining significant over the short scales 1–9. After a transition period, age-related complexity tended to decrease with negative regression (B values) at scales larger than about 15.

In the active state, the strongest complexity increase was found around scale 4, while remaining significant over the short scales 3–8. With regard to subsequent scales up to 20, the B values and significance almost decreased.

The significant short-scale complexity increase and tendency of long-scale complexity decrease in the quiet state as well as the short-scale complexity increase in the active state are shown in Fig. 5.

Since the complexity increased logarithmically with increasing scale (see Fig. 3, scale ≥2), this scale dependency was assessed by a robust MSE parameter that was designed as slope (regression line) of the exponential complexity increase over the range of scale 2 to scale 20 (Fig. 6). The clear slope decrease in the quiet state data reflects the relationship between the increase of short-scale complexity and the tendency of long-scale complexity decrease.

Power spectrum-based maturation parameters. In the quiet state, the relative contribution of HF and LF to total power significantly increased, but that of VLF to total power significantly decreased. In contrast, in the active state, these parameters were not found to be age related (Table 2).

**DISCUSSION**

The objective of the present work was to explore indices of maturating fetal autonomic control in the developing behavioral states.

In the developing quiet state, we found increasing formation of the vagal and sympathetic control that is reflected in the increasing part of HF and LF band powers and in the increasing complexity in the related short scales (MSE scales 1–9) of the heart rate fluctuations. The formation of heart rate patterns under the maturating vagal (decelerating) and sympathetic...

![Fig. 1. Typical fetal heart rate patterns (beat-to-beat follow up) showing increasing state differentiation with increasing gestational age (GA). Top traces: quiet state. Reduction of baseline fluctuations with increasing week gestational age (WGA). Bottom traces: active state. Formation of movement-related heart rate acceleration patterns with increasing WGA (movements not shown).](image)

![Fig. 2. Scatter plots and regression lines of histogram characteristics dependent on WGA and state.](image)
(accelerating) control is furthermore reflected in the shift from negative to positive skewness. The tendency of a decreasing long scale complexity might indicate an increasing functional protection and stabilization that approaches a quiet sleep heart rate pattern. As the strongest parameter, gMSE at scale 3 could explain 48% of the HRV via the fetal gestational age.

In the developing active state, multiple types of heart rate patterns appeared. These multiple internal coordinations and external influences become increasingly effective in the developing active state and lead to an increasing behavioral variability and render age dependencies less visible. It is furthermore obvious that the time expansion of the active state pattern limits their statistical evaluation from 10 and 30 min data sets, respectively. Nevertheless, the formation of heart rate acceleration patterns in association with increasing sympathetic activity and complex sympathovagal control is clearly reflected in the shift from negative to positive skewness that explains 27.9% (29.3%, 30-min interval) of HRV dependent on the fetal gestational age. In this connection, the SDNN also increased, however, less strongly. Furthermore, the short scale complexity increased at a lower rate, whereas the relative contribution of spectral rhythms did not change with age.

The skewness turned out as a strong index that might be related to the more pronounced development of sympathetic innervations (heart rate increases with slower time constant) compared with vagal innervations (heart rate decreases with faster time constant). In contrast, the previously used asymmetry index (16) based on information theoretic formulation of irreversibility considers the probability distributions of heart rate increases and decreases. It was less age related than the skewness in the present data and not reported here. It could be concluded that the increase of fluctuation amplitudes and heart rate acceleration patterns more strongly depend on the developmental age than their irreversibility and causality, respectively.

In summary, we were able to corroborate our hypotheses of increasing fluctuation amplitude (SDNN), pattern formation (skewness, frequency band distribution), and complexity increase at sympathovagal modulation scales with different degree of clarity in both states. Complexity decrease could only be indicated in the quiet state data. The previously reported complexity decrease in non-state-selected data sets can be explained by the increasing probability of appearing active states that show less complex heart rate patterns than the quiet states, such as also demonstrated in Fig. 3.

Table 2. Age dependencies of distribution (SDNN, skewness), complexity (gMSE), and power spectra density parameters in quiet and active state of 10-min data sets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Quiet (n = 111)</th>
<th>Active (n = 248)</th>
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<tr>
<td></td>
<td>R²</td>
<td>Slope (95% CI)</td>
</tr>
<tr>
<td>SDNN</td>
<td>0.066</td>
<td>0.07 (0.02, 0.12)</td>
</tr>
<tr>
<td>Skewness</td>
<td>0.204</td>
<td>0.01 (0.06, 0.13)</td>
</tr>
<tr>
<td>gMSE Scale 1</td>
<td>0.135</td>
<td>0.007 (0.004, 0.01)</td>
</tr>
<tr>
<td>Scale 3</td>
<td>0.48</td>
<td>0.011 (0.009, 0.014)</td>
</tr>
<tr>
<td>Scale 4</td>
<td>0.42</td>
<td>0.012 (0.009, 0.014)</td>
</tr>
<tr>
<td>Scale 20</td>
<td>0.043</td>
<td>−0.005 (−0.009, 0)</td>
</tr>
<tr>
<td>Slope scale 2–20</td>
<td>0.275</td>
<td>−0.002 (−0.003, −0.001)</td>
</tr>
<tr>
<td>PSD VL/TP</td>
<td>0.209</td>
<td>−0.012 (−0.017, −0.008)</td>
</tr>
<tr>
<td>LF/TP</td>
<td>0.191</td>
<td>0.009 (0.005, 0.012)</td>
</tr>
<tr>
<td>HF/TP</td>
<td>0.107</td>
<td>0.003 (0.001, 0.005)</td>
</tr>
</tbody>
</table>

Coefficient of determination (R²), regression line slope (95% confidence interval), significance (P < 0.001 in bold letters), strongest parameter per state are underlined. Additional results of 30 min active state data sets (n = 134) in italic letters.

In summary, we were able to corroborate our hypotheses of increasing fluctuation amplitude (SDNN), pattern formation (skewness, frequency band distribution), and complexity increase at sympathovagal modulation scales with different degree of clarity in both states. Complexity decrease could only be indicated in the quiet state data. The previously reported complexity decrease in non-state-selected data sets can be explained by the increasing probability of appearing active states that show less complex heart rate patterns than the quiet states, such as also demonstrated in Fig. 3.
If we suppose that the human being is the result of a natural self-organization and adaptation process and that ontogenetic development recapitulates phylogenetic development, universal rules and indices of developmental biology may be of real help to evaluate fetal maturation.

Indeed, we found respective HRV changes such as increasing variability and complexity as well as pattern formation. Consequently, these universal developmental characteristics provide appropriate measures of maturation. It can be concluded that these characteristics of reorganization (self-organization) and adaptation may also help to better understand and identify developmental disorders. This capability might be of significance in association with fetal programming of disturbances in later age due to adverse disturbances of the fetus. For example, attention-deficit hyperactivity disorders in adolescent boys were related to antenatal maternal anxiety (46) that potentially influences the development of fetal humoral and autonomic control reflected in heart rate patterns. Reorganization of autonomic control due to several systemic pathologies was associated with a systematic readjustment of complexity from different time scales in adults (12, 15) and may indicate the importance of considering scale-dependent adjustments in the prenatal diagnosis too.

The investigated time scales cover the whole range that includes the cardiogenic beat-to-beat excitation process, the vagal (HF) and sympathetic (LF) autonomic heart rate modulations, and long-lasting autonomic and humoral (VLF) control mechanisms. Approximately the same range was also covered by MSE, which allows a better assessment of complex interactions than the consideration of superimposed rhythms by PSD. The strongest age dependencies were not found in the cardiogenic rhythm at scale 1 but over a range of scales that reflect complex sympathovagal modulations. The statistical validity of MSE calculation from 10 min data was shown compared with simulated random signals and an additional 30-min data set. The dependency of the complexity estimators on the data distribution was essentially avoided by evaluating and comparing two established methods outlined in the Appendix. The gMSE uses quantile-based quantification of complexity that is per se robust against amplitude and distribution changes, whereas the rMSE considers changes of amplitude by normalizing on the standard deviation at each time scale. Both methods provided almost similar results.

There is a consensus that during fetal maturation, HRV increases, different fetal states emerge and develop, and the relative duration of active states increases. Since autonomic control follows different objectives during active and quiet behavior, a state-selective analysis potentially provides additional information relating to specific maturation aspects. Appropriate consideration of the developmental principles may facilitate early identification of developmental disturbances, and the possibility of designing innovative therapeutic strategies.

**Perspectives and Significance**

Epidemiological studies show that unfavorable prenatal disturbances can seriously affect health in later life. The phenomenon known as “fetal programming” resulting from adverse conditions...
influences to the fetus can be explained by epigenetic mechanisms. Effects related to fetal autonomic control are reflected in the heart rate pattern, which is the sole almost continuously available monitoring signal from the fetus during the essential developmental period across the second and third trimester. HRV was considered as an “order parameter” of the complex system “organism” and reasonably interpreted by universal indices of developmental biology.

With the use of the signal and system analysis methodology introduced herein, a new window for prenatal diagnosis in terms of the early detection of developmental problems may have been opened. The method may, furthermore, provide a basis for developing prophylactic and therapeutic strategies using the normal range of epigenetic plasticity during fetal maturation.

APPENDIX

Multiscale Entropy estimation

The validity of a multiscale complexity estimation from the 10-min heartbeat interval series was evaluated by comparing the sample entropy (SE) with the general mutual information (GMI)-based methodology and the results from analyzing simulated and the fetal study data sets.

Since complexity and resulting MSE estimation can be influenced by changing data length, data distribution, as well as the coarse graining methodology, the performance of two established methods was evaluated for the present data. Complexity estimated by SE depends on embedding dimension m, tolerance level r, and requires a large data sample (22). In the initially proposed MSE concept (5), the r value was set for each original data set and kept constant over all coarse graining scales. In this way, the coarse graining-related amplitude reduction was disregarded in favor of a constant calculation range. A subsequent comment by Nikulin and Brismar (30) and later work by Valencia et al. (45) proposed individual renormalization for each scale to avoid the dependency of SE estimation on the coarse graining-dependent amplitude decrease. The normalization of r in SE estimation with regard to SDNN might be the best approximation for keeping the complexity estimation independent of the data distribution in terms of variation amplitude. But in the present work, data were not normally distributed, and the skewness clearly depended on the gestational age. Hence, SE estimation might still be influenced by the distribution despite SDNN-based r value choice. This problem can be avoided by using quantile-based GMI calculation (35). The resulting gMSE estimation method is independent of the data distribution and hence also robust against outliers. A previous analysis of state independent fetal maturation provided almost similar results of gMSE and SE-based MSE using individual r values on each scale (16). The same holds for the present state-dependent fetal data. But remember that both methods may have different advantages and disadvantages depending on the particular data.

While the traditional coarse graining (5) effects a low-pass filter producing some spurious fast components, Butterworth low-pass filtering and resampling avoids these spurious components but reduces some other frequencies according to the filter characteristics (45). In the present work, the complete analysis was performed using...
both methods. The results showed no remarkable differences. Therefore, only the results of the filtering-based coarse graining method are presented.

\textit{rMSE} was calculated using \textit{SE} according to Richman and Moorman (38) using the Matlab code provided by Physionet (http://www.physionet.org/physiotools/sampen/matlab/) with embedding dimension \( m = 2 \) and tolerance range \( r = 0.15 \) standard deviation for each individual scale.

\textit{gMSE} was calculated by generalized mutual information [GMI, (34, 35)] using analysis data windows of 64 points, quartile partitioning (4 classes), and embedding dimension \( m = 1 \). The analysis windows were shifted with an overlap of 32 points over the scale-dependent data sets, and the entropy estimates of the windows were averaged to calculate a scale-dependent complexity. In the additional 30-min data sets, we used analysis windows of 128 points.

The essential agreement of the found \textit{gMSE} and \textit{rMSE} characteristics and age dependencies are shown in Fig. A1 vs. Fig. 3, and Table A1 vs. Table 2.

The decreasing data length with increasing scale is a relevant question when analyzing 10-min recordings. From an original data length of about 1,400 beat intervals, a data length of 70 results at coarse graining scale 20. Simulations using noncorrelated Gaussian white noise of the same data length show stable complexity estimates with slightly increasing confidence interval that, however, do not reach the range of maturation-related changes of interest (Fig. A1, Fig. 3). The different absolute values of \textit{gMSE} and \textit{rMSE} reflect their particular calculation. In an ideal random process, the \textit{gMSE} values calculated, for example, 4 bin partitioning, theoretically result in complexity estimates equal to 2 bit, here normalized into 1. This is nearly achieved if analyzing at least 1,024 data points. In the analyzed simulated 64 data points Gaussian white noise signal, the complexity is systematically underestimated leading to values of about 0.9. The short data length leads also to systematic underestimation of \textit{SE} according to Lake et al. (22).

The \textit{rMSE} dependencies on fetal age shown in Table A1 compared with the respective \textit{gMSE} results (Table 2) are more or less qualitatively accordant and confirm the validity of the 10-min data analysis. However, there is a tendency of lower correlation coefficients in the quiet state in contrast to higher correlation coefficients in the active state in \textit{rMSE} compared with \textit{gMSE}.

\textit{Complexity Over Scale and Maturation Assessment}

Linear functions well fitted the exponential entropy values on the scales between 2 and 20 (standard error of estimate \( \text{SEE} < 0.1 \)) in all of the individual recordings (for examples see Fig. A2). Consistent with the group average behavior according to Fig. A3, a significant positive slope can be supposed as predominant individual behavior. It was found in 96/111 (quiet, 10 min), 243/248 (active, 10 min), and 133/134 (active, 30 min) cases, respectively (see Fig. A3).

With the use of these cases only, the age-dependent regression statistics \( R^2 = 0.165, \text{slope} = -0.001(-0.002, -0.001), P < 0.001 \) (quiet, 10 min); \( R^2 = 0.04, \text{slope} = 0.0(-0.001, 0.0), P = 0.003 \) (active 10 min); and \( R^2 = 0.036, \text{slope} = 0.0(-0.001, 0.0), P = 0.029 \) (active 30 min) show minor changes compared with those of the entire data set (see Table 2). In the main analysis we did not remove the remaining cases since they may also reflect important age-dependent behavior.

\textbf{ACKNOWLEDGMENTS}

We thank Nasim Kroegel for language editing.

\textbf{GRANTS}

D. Hoyer and U. Schneider were supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, HO-1634 12-2, Schn 775/2-3).

\textbf{DISCLOSURES}

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

REFERENCES


