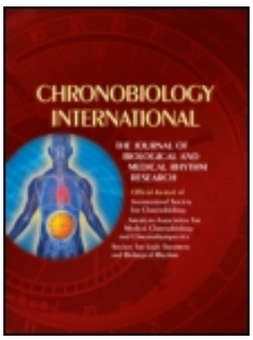

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ORIGINAL ARTICLE

Influence of acoustic stimulation on the circadian and ultradian rhythm of premature infants

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The aim of the present study was to evaluate the development of the circadian rhythm of the salivary cortisol in premature infants and its correlation with the onset of the sleep–activity behavior pattern during the first 3 weeks of life under controlled light:dark conditions. Furthermore, we investigated the influence of acoustic stimulation by audiotaped lullabies or the maternal voice on the cortisol values and long-term sleep–activity patterns. The study was a block-randomized, prospective clinical trial with a study population of 62 preterm neonates (30 < 37 gestational age). We compared two study groups who listened either to music or to the maternal voice (music: $N = 20$; maternal voice: $N = 20$) with a matched control group ($N = 22$). The acoustic stimulation took place every evening between 20:00 and 21:00 h for 30 min over a period of 2 weeks. The cortisol values and activity–rest behavior of the neonates were determined during the first 3 weeks of life on the 1st, 7th and 14th day. Actigraphic monitoring was used to record the activity pattern continuously over 24 h and a validated algorithm for neonates was used to estimate sleep and wakefulness. The saliva samples were obtained 10 min before and 10 min after the acoustic interventions for the study groups. Additionally, saliva samples were obtained from the control group seven times over a 24-h period (20:00, 21:00, 01:00, 05:00, 08:00, 13:00 and 17:00 h). The cortisol data were analyzed by fast Fourier transformation to assess periodic characteristics and frequencies. Hierarchical linear modeling was further performed for the statistical analysis. *Results:* The cortisol rhythm analysis indicated a circadian rhythm pattern for only one premature infant, all others of the neonates showed no circadian or ultradian rhythm in cortisol. Cortisol level of the premature neonates was significantly higher during the first day of the study period at night-time (median: 17.1 nmol/L, IQR = 9.7–24.4 nmol/L) than on days 7 (median: 9.6 nmol/L, IQR = 4.7–14.6 nmol/L; Tukey-HSD, $z = 4.12$, $p < 0.001$) and 14 (IQR = 5.8–13.7 nmol/L; Tukey-HSD, $z = 2.89$, $p < 0.05$). No significant effect of acoustic stimulation was observed on the cortisol concentration and sleep–wake behavior. The activity–sleep rhythm of preterm neonates was dominated by ultradian rhythm patterns with a prominent period length of 4 h (30.5%). Activity frequencies of neonates were also significantly higher overnight on the first study day (mean: 329 ± 185.1 U) than of night seven (mean: 260.2 ± 132.4 U; Tukey-HSD, $z = 2.50$, $p < 0.05$). Quiet-activity patterns increased, whereas high-activity patterns decreased during the observation period. Average sleep time increased significantly during the study time from day 1 to day 7 (Tukey-HSD, $z = 2.51$, $p < 0.05$). In conclusion, premature infants showed higher cortisol levels – without a circadian rhythmicity – and higher activity frequencies in the first days after birth which may reflect an adaptation process of neonates after birth. Cortisol concentrations and the activity patterns were not influenced by music interventions.

Keywords: Actigraphy, activity, music intervention, preterm, rhythms, salivary cortisol, sleep

INTRODUCTION

The circadian system is coordinated in mammals by the suprachiasmatic nuclei of the anterior hypothalamus (SCN) (Moore & Eichler, 1972; Stephan & Zucker, 1972). The ontogeny of the circadian system concerning the SCN, afferent and efferent neuronal pathways as well as the biochemical and physiological factors have been extensively studied in animal models (Moore, 1973; Reppert & Schwartz, 1983). In human fetus, day–night rhythms have been detected in heart rate, respiratory rate and adrenal corticosteroids (Seron-Ferre et al., 1993, 2001). However, the fetal circadian system is mainly synchronized by the maternal circadian rhythm; the neonate loses this synchronized circadian

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rhythmicity after birth and needs some time to adapt his own circadian rhythmicity to the extra-uterine life (Mendez et al., 2012). Day–night rhythms in melatonin and cortisol are generally detected between the 3rd and 6th months of age (Kennaway et al., 1992). Exposure of premature infants to cycling light results in earlier establishment of, e.g. rest-activity 24-h rhythm (Rivkees et al., 2004), whereas continuous lighting may adversely affect the development of this circadian rhythm (Antonucci et al., 2009), demonstrating that the development of the circadian systems needs light stimulation. Light is beneficial for infant entrainment of circadian rhythms (Tsai et al., 2012). In general, preterm infants have a higher risk of peri- and/or postnatal diseases (e.g. cerebral hemorrhage, perinatal asphyxia, respiratory distress syndrome (RDS) or pneumothorax) (Boyle et al., 2012; Maier & Obladen, 2011). In addition, preterm infants are exposed early to medical and caregiving interventions. These unique circumstances of preterm infants are hard to simulate in animal models (Glotzbach et al., 1995). However, further awareness and analysis of the development of the circadian system in hospitalized preterm infants is an important issue as stable circadian rhythms are essential for health and survival (Glotzbach et al., 1995; Moore-Ede et al., 1983) and have a great influence on the effectiveness of medications and interventions as shown in adults and children (Lemmer, 2006; Potts et al., 2011; Redfern & Lemmer, 1997).

The steroid hormone cortisol is regulated by the hypothalamus–pituitary–adrenal (HPA) axis in humans. Cortisol adapts the physiological parameters of the body to stressful stimuli. In adults, cortisol shows pulsatile secretions with high values early in the morning and lowest concentrations at around midnight (endogenous rhythm). Exogenous stimuli (e.g. stress) stimulate the cortisol secretion and create great fluctuations of the cortisol level (Behrends et al., 2012). Whereas older studies due to less data/24 h and less-sensitive methods of analysis gave inconclusive results on the maturation of entrained cortisol rhythm, recent studies analyzing a greater number of blood/saliva samples within 24 h indicate that a stable circadian rhythm in cortisol is achieved only after ~2–4 months after birth (Antonini et al., 2000; de Weerth et al., 2003; Iwata et al., 2013).

The sleep–wake behavior of neonates was examined in earlier studies on sleep diary information collected by parents and study nurseries. More recent studies have used actigraphic monitoring methods to describe longitudinal assessments in activity–rest patterns of infants (Korte et al., 2001; Nishihara et al., 2002) as the data of actigraph monitors are more reliable and provide higher statistical power for analysis (Ancoli-Israel et al., 2003; Sadeh, 2011; Sadeh & Acebo, 2002). Previous studies demonstrated that ultradian rhythms of various period lengths dominated the heart rate, blood pressure, skin temperature, activity and cortisol profiles of neonates in the first week of life (Glotzbach et al., 1995; Korte et al., 2001; Mirmiran & Kok, 1991). It has been suggested that environmental influences (''Zeitgeber'') as light–dark cycle, food ingestions, sleep–wake cycles and social cues are major determinants in synchronizing human circadian rhythm (Aschoff 1954; Aschoff & Wever, 1976; Halberg, 1963; de Weerth et al., 2003). The emergence of an entrained circadian rhythm indicates the maturation of the central nervous and the circadian timing system with clock mechanisms driving the endogenous oscillators. In line of this suggestion is the observation that the activity rhythm of full-term infants shows earlier prominent 24-h components than the activity patterns of preterm infants (Korte et al., 2001).

Moreover, the sensory experiences and acoustic stimulations of preterm infants in the newborn intensive care unit (NICU) vary greatly from the acoustic environment of the fetus at the same gestational age. *In utero*, the melody and sound of the mother's voice as well as the rhythm of the mother's heart are prominent. Several studies describe that preterm infants benefit from acoustic stimulation with lullabies or maternal voice by showing a decrease of heart rate, an increase of oxygen saturation levels, a faster gain of weight and a shorter time of discharge from hospital (Caine, 1991; Loewy et al., 2013; Standley, 2002; Standley & Moore, 1995). Parental voice also seems to improve the development of speech and language processing in premature infants (Caskey et al., 2011; Krueger, 2010). However, only little is known about the influence of musical and vocal stimulation on the cortisol levels and on the long-term activity behavior of premature infants. The present manuscript is part of an investigation in premature infants in whom the effects of lullabies or maternal voice was studied on several physiological functions such as heart rate (Wirth et al., unpublished), cortisol and activity.

We initiated this study because of the great variance of results concerning the development of circadian cortisol and activity–rest rhythm of preterm infants. An additional stimulus was the observation of one of the authors that different kinds of music were shown to significantly affect cardiovascular functions in rats (Lemmer, 2008). A further purpose of this study was to reach a better understanding on the first processes of adaptation concerning the cortisol and activity–rest behavior of preterm infants during the first 3 weeks of life. We measured salivary cortisol seven times over 24-h and monitored the activity by using actigraphy to test our hypothesis of whether preterm infants adapt to circadian patterns during the first weeks of life or ultradian rhythm predominates. Furthermore, we investigated how acoustic stimulation by audiotaped lullabies or the maternal voice influences the development of circadian activity rhythm and cortisol levels.

METHODS

Study population

Sixty-two premature infants were randomly selected from those admitted to the newborn intensive care unit (NICU, I7) and the neonatal unit (042) of the Philipps University Hospital at Marburg, Germany. Infants were monitored from 1 March 2012 to 21 January 2013. They were monitored in incubators with 10 h of light during daytime [mean: 756 ± 601 lx (I7); mean: 380 ± 26 lx (042)] and dim light [mean: 5 ± 13 lx (I7); mean: 7 ± 12 lx (042)] during night. Only preterm infants with the following criteria were included: gestational age between 30 < 37 completed weeks and a postnatal age of <7 days. Exclusion criteria were: mechanical ventilation at the time of data collection, major malformations, major illnesses, withdrawal of consent, auditory defect. The parents of the infants participated voluntarily in the study and were contacted through the clinical specialists of the hospital. The project was approved by the local Medical Ethics Committee and parental written consent was obtained. Baseline information obtained from each infant included gestational age, sex, birth weight, postnatal age and antenatal betamethasone therapy. All clinical data were pseudonymized.

Study design

The study was designed as a block-randomized, prospective clinical trial. The randomization was generated in R 2.15.0 (R Development Core Team, 2008) by using the ''blockrand'' package (Snow, 2006). The patients were then randomly selected by picking a closed envelope for each study group (e.g. music, maternal voice or control). The whole study period lasted 14 days. The infants of the two study groups (music, maternal voice) received 30 min of the acoustic intervention every evening between 20:00 and 21:00 h. The patients of the music group listened to a collection of lullabies (Kaufmann et al., 2009). The maternal voice group listened to a piece of the book ''The little prince'' (Saint Exupéry de, 1995) read by his/her mother, whereas the maternal voice was recorded before the beginning of the study with a voice recorder (IC recorder Sony ICD PX 312, Sony GmbH, Germany) and further transferred to an audio-CD. The acoustic interventions were provided by an audio-player (UR 1305, Kracher, Germany) and loudspeaker (Ricco P11 mini USB, Ricco Europe Co., UK) which were placed in the corner of the neonate's incubator. The acoustic stimulation time was not disturbed by visitors, caregiving or medical procedures. The volume of the music applications was regularly controlled before and during the acoustic interventions by a sound level meter (MS-67 Pro Digital Sound Level Meter, ELV Elektronik AG, Germany) to ensure that the acoustic stimulation stayed in the recommended range of 55–65 dB (Philbin & Klaas, 2000). Furthermore, the environmental sound level was recorded and controlled during the stimulation period. The control group received no acoustic stimulation.

Data collection of saliva samples

Saliva samples were obtained from the two intervention groups (music, maternal voice) and the control group 10 min before and 10 min after the acoustic stimulation on the 1st, 7th and last day of the study period. Additionally, saliva samples from the control group were taken at seven time points over a 24-h period 20:00, 21:00, 01:00, 05:00, 08:00, 13:00 and 17:00 h on days 1, 7 and 14 of the study. The saliva was collected non-invasively by standard universal cotton swabs (NOBA Danz GmbH, Germany) which were placed in the infant's mouth in contact with the inner cheek for 5–10 min. We used no salivary stimulants and ensured that the saliva was taken before the infants' feedings to avoid contaminations of the samples with milk. Mothers and study nurses confirmed that the babies did not demonstrate any signs of distress during this procedure. Basal plasma and salivary cortisol values have been shown to be well correlated ($r = 0.48$–0.67) (Calixto et al., 2002; Chou et al., 2011). Additionally, salivary cortisol reflects the free part of cortisol which cannot be affected by plasma binding protein concentrations and saliva flow rate does not affect cortisol levels (Neu et al., 2007). The collected samples were refrigerated at 6 °C for 1–5 days prior to centrifugation. Each of the swab ends were then placed in a prepared Eppendorf tube (Sarstedt AG & Co, Germany) (Figure 1) and were further placed in a Cellstar tube (Greiner Bio-One GmbH, Germany) for centrifugation. After centrifugation for 30 min, the saliva was pipetted from the bottom of the tube and stored in a labeled tube (Greiner Bio-One GmbH, Germany). All samples were frozen and stored at −20 °C until

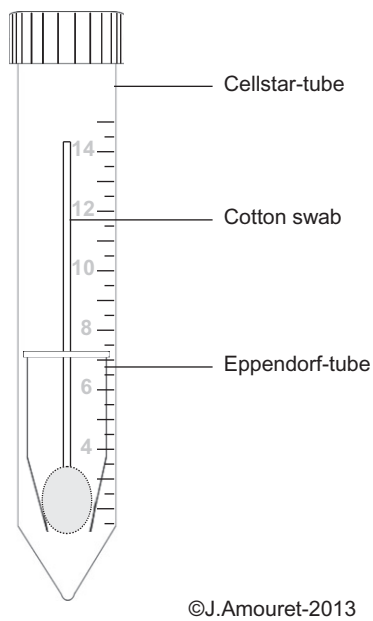

FIGURE 1. Method of extracting saliva of the collected cotton swabs.

analysis. Salivary cortisol was determined by competitive ELISA (enzyme linked immune sorbent assay). The samples were measured by using a commercially available ELISA-kit ''RE 52611'' (IBL International GmbH, Germany) according to the given instructions. Only samples containing at least 50 μL of saliva were analyzed. The commercial controls for high and low cortisol concentrations were determined in every plate for calculating the inter- and intra-assay coefficients of variability and were controlled against the acceptable range given by the producer. In addition, the light intensity on the neonatal ward was measured throughout a 24-h period (Luxmeter Mavolux 5032 C, Gossen GmbH, Germany).

Monitoring of the activity–rest behavior

The rest–activity behavior of the premature infants was measured using actigraphic monitoring devices (ActiSleep+, ActiGraph, Pensacola, FL). The actigraph is a wristwatch-like activity monitor with the dimensions of 46 mm × 33 mm × 15 mm and a weight of 19 g, which was attached at the infant's ankle. Vertical, horizontal and perpendicular axes activity acceleration data are collected as raw data with a sample frequency of 30 Hz. For activity and sleep analysis the raw data are filtered and accumulated in 60-s epochs and further analyzed using the ActiLife software version 6 (ActiGraph, Pensacola, FL). The activity behavior of the preterm neonates was recorded over 24 h on the 1st, 7th and last day of the study. Interruptions of the data analysis due to medical interventions, technical problems or discharge of the infants were documented in the protocol. It was not possible to exclude artifacts caused by intense passive movements (e.g. feeding and caregiving procedures as well as small medical interventions) as parallel recordings of rest–activity diaries during the study period could not be achieved.

Statistical analysis

The descriptive statistics on demographic data, treatments and results are presented as median (interquartile range), mean (standard deviation) or number (percentage).

The circadian and ultradian rhythm analysis was performed with the program Chronos-Fit Version 1.06 (Zuther et al., 2009). The program Chronos-Fit performs a partial Fourier transformation and a stepwise regression technique of the data which fits each harmonic separately and checks the significance by *F*-test for each. The period of night started at 20:00–07:00 h and the daytime between 07:00 and 20:00 h which depended on the daily routine at the NICU and neonatal unit. The cortisol data were further analyzed with linear mixed-effects models (Hierarchical Linear Modelling) by using the package ''LME4'' implemented in R software (Bates et al., 2014). The mixed-effects models were used to determine the association between saliva cortisol levels and the different times of the day and study days. A stepwise selection procedure which consisted of a ''forward addition'' enabled to adjust for the individual longitudinal random factors. The selection was guided by the goodness-of-fit criterion-Akaike Information Criterion (AIC). It is considered that the model with the lowest AIC has the best prediction for a data set (Lai et al., 2006). The forward addition step procedure started with an empty model, which consisted only of the fixed effect term and the random individual effect. The covariates were then added one-by-one to the model and the optimal fitted model with the lowest AIC was chosen. The fixed effects were ''study group'' (music, maternal voice, control), ''study day'' (days 1, 7 and 14) or ''time of sampling'' (20:00, 21:00, 01:00, 05:00, 08:00, 13:00 and 17:00 h). The random effects were the individual effect and the study day effect. Due to the distribution of the residuals for each selected model, the base 10 logarithm of cortisol was carried out to normalize the data. A two-way analysis of variance with repeated measures was carried out on the optimal model. Significant *F* ratios from the analyses were followed by pairwise comparisons by using Tukey-HSD (honestly significant difference) test. Furthermore, the effect of the acoustic stimulation was evaluated by computing the difference between the cortisol level before and after the acoustic intervention. The data were further analyzed with the above-mentioned linear mixed-effects models.

The sleep and activity was analyzed using the ActiLife Program Version 6 (ActiGraph, Pensacola, FL). Therefore, the raw activity data were filtered and accumulated into 60-s epoch-level data and validated for wear time. Gaps of inactivity for more than 120 min, when no acceleration in all of the three axes appeared, were exclude from further analysis. No defined algorithm to analyze the sleep and activity pattern of preterm infants is described in the literature. For that reason, we used the algorithm of So et al. (2005), which was developed for neonates of an age between 2 and 4 weeks. The intensity of activity is divided into three categories. The first category consists of all low-activity frequencies <37 counts/min, the second category describes moderate-activity frequencies between 38 and 419 counts/min and the last category defines a high-activity level with all activity counts >420 counts/min. Furthermore, we analyzed the development of the sleep time by calculating the sleep efficiency, which is the registered sleep duration divided by the total time of the recorded activity frequencies computed for every infant separately. Statistical analysis of the rest–activity parameters were performed with a two-way ANOVA with repeated measures and a Tukey-HSD by using a general linear mixed model (GLMM) with the package ''nlme'' implemented in R software (Pinheiro et al., 2014). Interactions between the cortisol values, activity and the gestational/postnatal age, sex and antenatal betamethasone therapy were assessed with the analysis of regression by using linear mixed-effects models.

All calculations and statistical procedures were carried out in Microsoft Excel 2010 (Redmond, WA), SPSS Statistical Software Version 21.0 (SPSS Inc., Chicago, IL; 1984) and R software version 2.15.0. The threshold value for significance was $p < 0.05$.

RESULTS

Anthropometric variables

During the study period, data for 2 of the 62 randomized patients were removed from the analysis due to withdrawal of the parents' consent. Seven infants were discharged from hospital before the end of the study period and one neonate died during participation. Hence, data for these eight infants were analyzed only partially (Figure 2). The anthropometric and clinical characteristics of the 60 analyzed infants are similar and are summarized in Table 1. The mean gestational age of the infants are 33 + 0 weeks, the mean of the birth weight is 1840 g (SD = 520 g), the mean postnatal age 4.9 days (SD = 2.2) and the median of the 5-min Apgar scores are 8 (range = 5.7–10.3). The sex ratio of the study group was balanced with 53 % male ($N = 31$) and 47 % ($N = 29$) female participants.

Cortisol rhythm patterns

The circadian cortisol rhythm was analyzed for 20 premature infants from the control group. About 65% ($N = 274$) of the samples contained an adequate volume (approximately 25 μL) for the detection sensitivity of the enzyme-immune assay and therefore results could be obtained. The reason for the inadequate amount of saliva from 35% ($N = 148$) of the participants was most likely due to a dry mucous of some premature infants and to evaporative loss after the freeze-thaw process required for sample storage.

Salivary cortisol concentrations of all infants studied are summarized in Table 2. The rhythm analysis with Chronos-Fit indicated for only one premature infant a circadian rhythm pattern on day 1. This infant was born at 33 + 6 weeks of gestation and included in the study 3 days after birth. All other infants showed no circadian

FIGURE 2. Study flow chart of the trial.

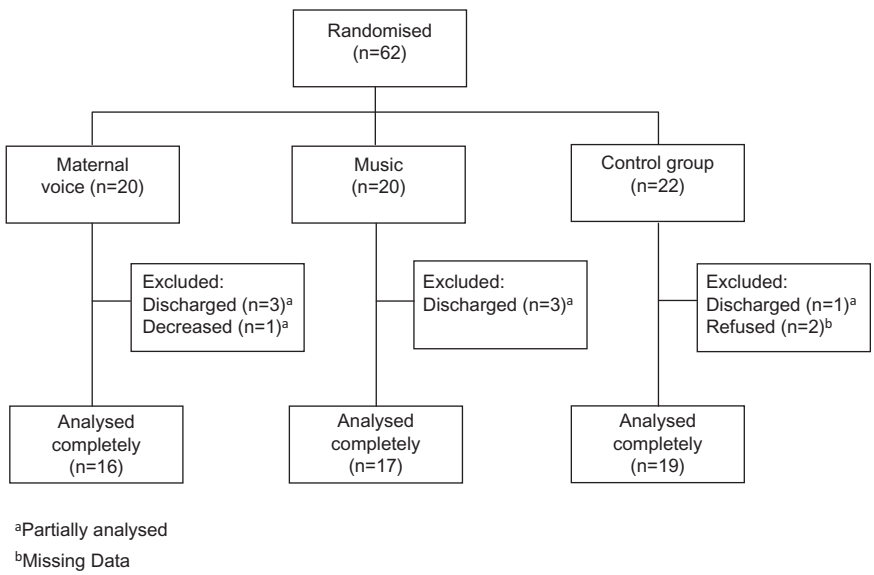

TABLE 1. Perinatal characteristics of the studied preterm neonates.

		All infants ($N = 60$)	Music ($N = 20$)	Maternal voice ($N = 20$)	Control ($N = 20$)
GA (weeks)	Mean (SD)	33 + 0 (10.8)	33 + 1 (11.7)	32 + 3 (11.9)	33 + 4 (7.2)
PNA (days)	Mean (SD)	4.9 (2.2)	4.7 (2.1)	6 (2.2)	4.1 (2)
Sex (%)					
Male		31 (53%)	12 (60%)	11 (55%)	8 (40%)
Female		29 (47%)	8 (40%)	9 (45%)	12 (60%)
Birth weight (g)	Mean (SD)	1840 (520)	1850 (545)	1691 (568)	1979 (417)
APGAR 5′	Median [Min–Max]	8 [3–10]	8 [3–10]	9 [5–10]	9 [5–10]
APGAR 10′	Median [Min–Max]	9 [4–10]	9 [4–10]	9 [6–10]	10 [6–10]
Associated disorder[a] [%]		5 [8]	2 [10]	3 [15]	0 [0]
CPAP [%]		9 [15]	3 [15]	3 [15]	3 [15]

[a]Necrotizing enterocolitis, intracranial hemorrhage and sepsis.

N, study population; SD, standard deviation; Min, minimum; Max, maximum; GA (weeks), gestational age at births; PNA (days), postnatal age at study beginning; APGAR 5′, APGAR score after 5 min; APGAR 10′, APGAR score after 10 min; CPAP, continuous positive airway pressure.

TABLE 2. Sequential salivary cortisol measurements (nmol/L) sampled seven times per day ($N = 19$).

	Day 1			Day 7			Day 14		
Time	Median	Range	*n*	Median	Range	*n*	Median	Range	*n*
20:00	19.4	[9.5–50.5]	15	9.8	[4.9–27.0]	15	10.1	[4.9–31.4]	12
21:00	16.7	[4.1–45.0]	14	8.1	[3.2–65.8]	14	9.5	[5.5–50.0]	12
01:00	21.9	[9.7–119.7]	11	9.9	[3.8–37.0]	14	9.5	[5.2–28.2]	12
05:00	12.4	[5.5–66.5]	14	9.9	[4.8–23.9]	12	12	[5.2–37.3]	11
08:00	14.9	[4.1–36.5]	10	14.4	[4.9–43.0]	15	8.2	[4.5–21.2]	11
13:00	13.9	[5.9–73.5]	16	10.7	[5.4–36.1]	12	12.8	[3.1–30.6]	8
17:00	11.8	[5.3–68.9]	15	9.7	[5.4–11.8]	7	11.1	[6.9–27.4]	12

n, number of measurements.

TABLE 3. Parameter estimates from the chosen hierarchical linear modeling of the infants' cortisol data.

Fixed effects	Estimate	SE	*p* Value
Intercept (Day 1)	1.23	0.04	
Day 7	−0.20	0.05	<0.001
Day 14	−0.17	0.05	<0.01
Intercept (Day 14 night)	1.06	0.05	
Day 1 night	0.18	0.06	<0.01
Day 7 night	−0.07	0.06	0.302

SE, standard error.

or ultradian rhythm for cortisol in the first 3 weeks of life. The cortisol levels showed intra- and interindividual variability. Furthermore, the results from the linear mixed model indicated that the factor day was significantly relevant suggesting that the cortisol values changed during the measured period by considering all premature infants (Table 3). The median cortisol concentration on day 1 (median: 16.5 nmol/L, IQR [interquartile-range] = 10.6–27 nmol/L) was significantly higher than on days 7 (median: 9.8 nmol/L, IQR = 6.3–16.4 nmol/L; Tukey-HSD, $z = 3.90$, $p < 0.001$) and 14 (median: 9.1 nmol/L, IQR = 7.6–15.5 nmol/L; Tukey-HSD, $z = 3.03$, $p < 0.01$) (Figure 3). The comparison between day and night cortisol levels showed that the cortisol concentration of day 1 was particularly high during the night-time. The median cortisol value of night one (median: 17.1 nmol/L, IQR = 9.7–24.4 nmol/L) was significantly higher than on night 7 (median: 9.6 nmol/L, IQR = 4.7–14.6 nmol/L; Tukey-HSD, $z = 4.12$, $p < 0.001$) and on night 14 (median: 9.8 nmol/L, IQR = 5.8–13.7 nmol/L; Tukey-HSD, $z = 2.89$, $p < 0.05$) (Figure 4). No statistically significant correlation could be demonstrated between salivary cortisol concentration and gestational age, postnatal age, birth weight, sex or prenatal betamethasone exposure.

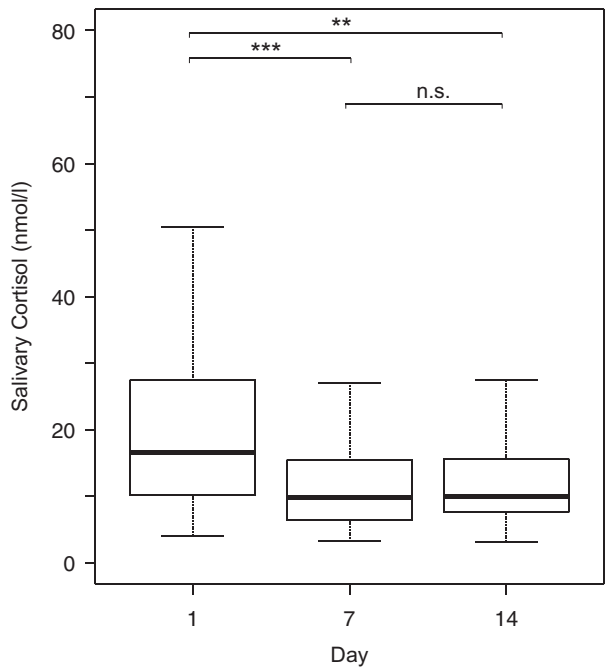

FIGURE 3. Pooled results of the salivary cortisol measurements of premature infants on day 1 ($N = 95$), 7 ($N = 89$) and 14 ($N = 78$). Boxplots represent 10th, 25th, 50th, 75th and 90th percentiles. $^{**}p < 0.01$, $^{***}p < 0.001$, n.s., non-significant.

Activity rhythm patterns

The results of the activity rhythm analysis were obtained from 83% measurements of the 50 premature infants included (151 out of 180 measurements). Some data sets were not registered completely over 24 h due to technical problems and medical interventions. The majority of the premature infants showed no circadian rhythm in the first 3 weeks of life. A circadian rhythm pattern was seen only in 5 out of 56 infants on day 1, 5 out of 47 infants on day 7 and 1 out of 48 infants on day 14 of the study period. Ultradian rhythm patterns with different lengths were mainly present in the premature infants, whereas the period length of 4 h dominated within all infants studied ($N = 46$, 30.5%), (Table 4). Comparing day and night-time activity frequencies for all of the subjects, the mean activity frequency on night one (mean: 329 ± 185.1 U) was significantly higher than on night seven (mean: 260.2 ± 132.4 U; Tukey-HSD, $z = 2.50$, $p < 0.05$) but not higher than on the 14th night (mean: 273.2 ± 130.2 U). No distinct difference was observed between the 7th and 14th night. However the mean daytime activity components did not change significantly over the study period (Figure 5). The average activity frequencies of day and night-time decreased significantly at increasing postnatal age (ANOVA, d$f = 1$,

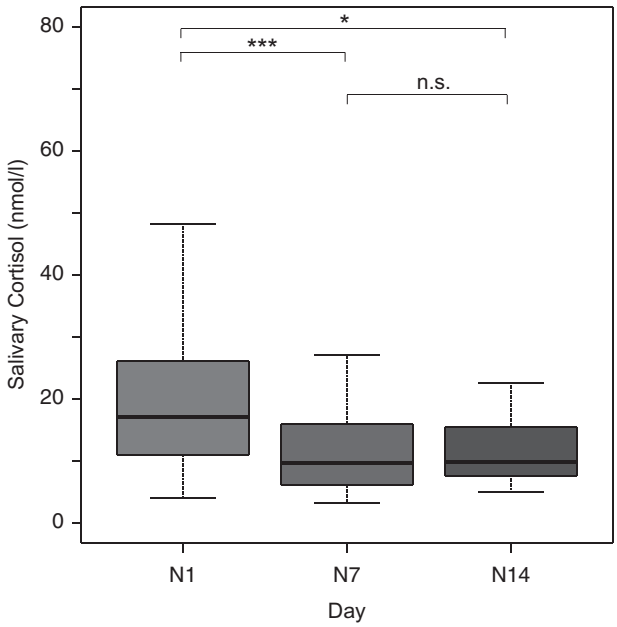

FIGURE 4. Pooled results of the salivary cortisol measurements of premature infants on night one ($N=54$), 7 ($N=55$) and 14 ($N=47$). Boxplots represent 10th, 25th, 50th, 75th and 90th percentiles. *$p<0.05$, ***$p<0.001$, n.s., non-significant.

TABLE 4. Activity rhythm analysis.

PL (h)	All days ($N=151$)	Day 1 ($N=56$)	Day 7 ($N=47$)	Day 14 ($N=48$)
4	46 (30.5%)	15	14	17
4,8	21 (13.9%)	10	6	5
6	34 (22.5%)	11	16	7
8	18 (11.9%)	9	3	6
12	20 (13.2%)	6	3	11
24	12 (7.9%)	5	5	2

PL, period length measured in hours; *N*, study population.

$p<0.01$). The gestational age and sex had no significant influence on the activity behavior.

Sleep and wake behavior

The activity behavior could be analyzed with the ActiLife program on 85% (153 out of 180 measurements) of the collected data. On an average, premature infants spent more time in the quiet (low) activity level (median: 15:54 h) than in the middle (median: 05:17 h; Tukey-HSD, $z=13.6$, $p<0.001$) and high activity level (median: 04:26 h; $z=16.3$, $p<0.001$) during the days of registration. On the other hand the infants' sleep–wake behavior changed with advancing age. The quiet activity bouts of the premature infants gradually increased from day 1 (54.8%) up to day 7 (61.1%; Tukey-HSD, $z=-3.33$, $p<0.01$) and day 14 (60.2%; Tukey-HSD, $z=-2.55$, $p<0.05$). In contrast, the mean percentages of the middle activity frequencies decreased progressively from day 1 (24.4%) to the 7th (21.1%; Tukey-HSD, $z=2.58$, $p<0.05$) and 14th day (21.2%; Tukey-HSD, $z=2.95$, $p<0.01$) of the study period. The mean percentages of the high activity patterns decreased slightly as well but did not differ significantly (day 1: 20.7%; day 7: 17.9%, $p=0.126$; day 14: 18.6%, $p=0.092$) (Figure 4).

Additionally, the average percentage of sleep efficiency time increased significantly from day 1 (66.1%) to day 7 (72.7%; Tukey-HSD, $z=2.51$, $p<0.05$) but increased not significantly compared to day 14 (69.3%; Tukey-HSD, $z=1.25$, $p=0.406$) (Figure 5).

Effects of acoustic stimulation on the activity cycles and the hormone cortisol

The activity patterns of the preterm infants were not different between the music, maternal voice or control group (Figure 6). Furthermore, the sleep and wake behavior was not significantly influenced by the acoustic stimulation (Figure 7). The measured cortisol levels were not modified 10 min after the auditory music or maternal voice stimulus (ANOVA, d$f=2$, $p=0.42$). The average noise level of the environment was 48 dB on the neonatal intensive care unit and 55 dB on the neonatal unit.

DISCUSSION

Circadian and ultradian rhythm

Our results showed no stable circadian pattern of cortisol in preterm infants during the first 3 weeks of life. A circadian cortisol rhythm was only found in one premature infant on day 1 of the study period. This is in accordance with data from Kidd et al. (2005) who found rarely a circadian rhythm in cortisol in preterm infants before 30 weeks' of gestation.

Previous studies described the appearance of circadian rhythm by the age of 3–6 months (Kennaway et al., 1992; Vermes et al., 1980), whereas more recent articles have observed circadian pattern in premature infants already between 2 and 8 weeks of age (Antonini et al., 2000; Castro et al., 2000; Herrington et al., 2004; Santiago et al., 1996; Tollenaar et al., 2010). The lack of agreement in these studies is probably due to differences in the sampling times and frequencies, as some studies took only samples in the morning and in the evening of a 24-h period (Antonini et al., 2000; Castro et al., 2000; Kidd et al., 2005). Consequently they defined the appearance of the circadian rhythm as a higher value in the morning than in the evening (Krieger et al., 1971) which is not in agreement with the definition of the term circadian (Halberg, 1963). Considering the episodic secretion pattern of cortisol in humans, a regular rhythmic pattern cannot be defined with two samplings per day (van Cauter et al., 1996; Vermes et al., 1980). Furthermore, the statistical methods in analyzing the data differed between the previous studies. Consequently, it is shown that the wide variance of the age of appearance of the circadian rhythm is due to different analytical working definitions and low sample

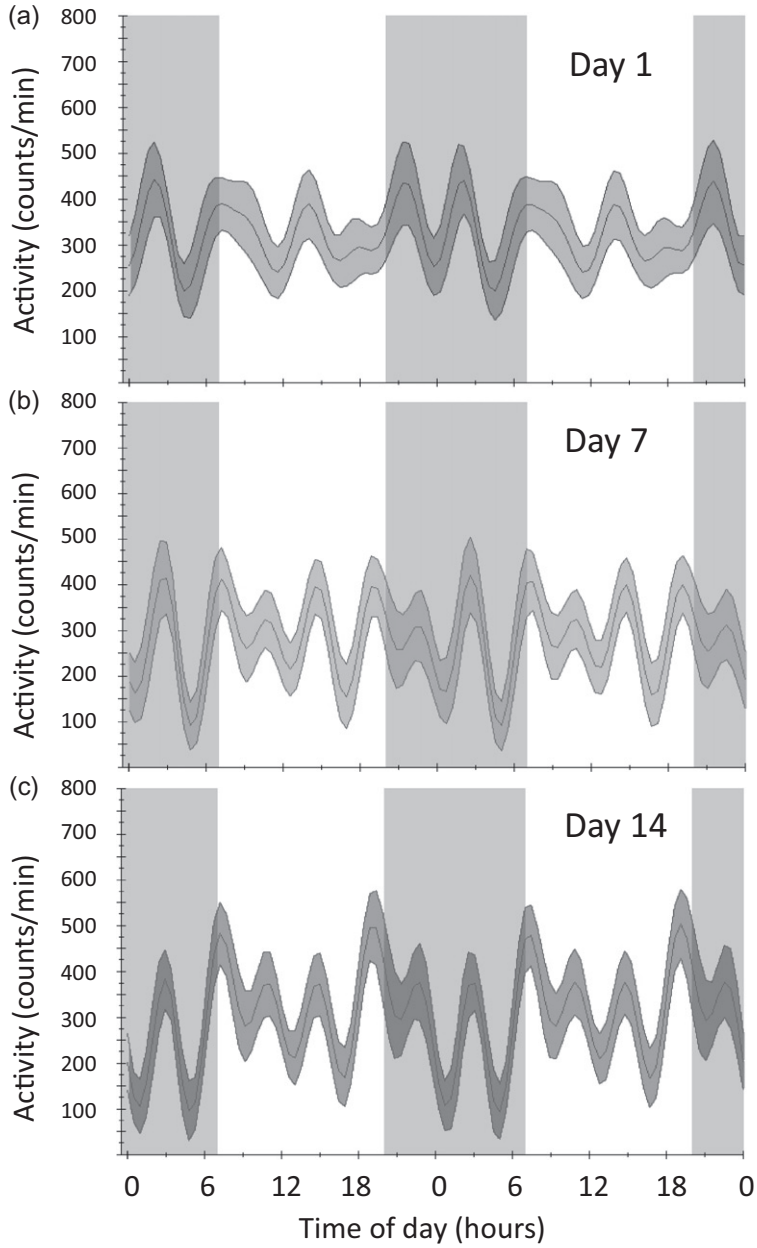

FIGURE 5. 24-Hour profiles of the activity behavior of preterm infants on (a) day 1 ($N=56$), (b) 7 ($N=47$) and (c) 14 ($N=48$). The data were fitted with Chronos-fit (Zuther et al., 2009). Group mean values are plotted with their respective 95% confidence intervals. The white background indicates the daytime and the grey background the night-time.

frequencies (Antonini et al., 2000; Krieger et al., 1971; Price et al., 1983; Santiago et al., 1996; de Weerth et al., 2003). Circadian patterns of physiological parameters like body temperature and heart rate frequency are already developed in preterm and full-term neonates during the first week of life (Glotzbach et al., 1995; Mirmiran & Kok, 1991). Although the HPA axis of preterm and term neonates showed normal ACTH-secretions after Metyrapone-tests and normal cortisol responses after ACTH-injections (Battin et al., 2007; Bettendorf et al., 1998; Kari et al., 1996), experimental animal studies have shown that suprahypothalamic brain structures are important for the maturation of circadian cortisol patterns (Moore & Eichler, 1972). Consequently an immature central nervous system in neonates could be one reason for the delayed circadian development (Vermes et al., 1980).

The present study demonstrated that the cortisol levels of the premature infants were significantly higher in the first 7 days of life compared to the subsequent study days. The cortisol concentration of the premature infants was particularly higher overnight during the first postnatal days. Previous studies in preterm infants showed similar results with a higher baseline cortisol level in the first weeks of life which gradually decreased in the following weeks (Doerr et al., 1988; Economou et al., 1993; Kidd et al., 2005; Metzger et al., 1993; Scott & Watterberg, 1995; Vermes et al., 1980). Vermes et al. (1980) and Kidd et al. (2005) found increased cortisol concentrations of the preterm infants up to the first 3–4

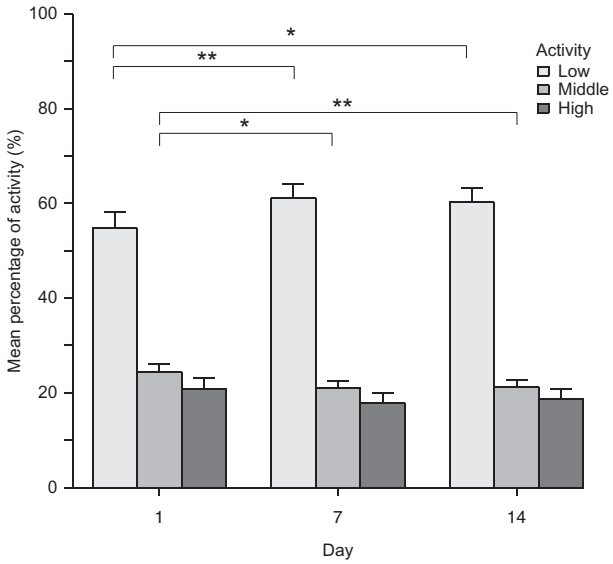

FIGURE 6. Development of the activity patterns of preterm infants is illustrated as mean averages of the three activity levels low, middle and high per study day (day 1: $N = 56$, day 7: $N = 49$, day 14: $N = 48$). *$p < 0.05$; **$p < 0.01$.

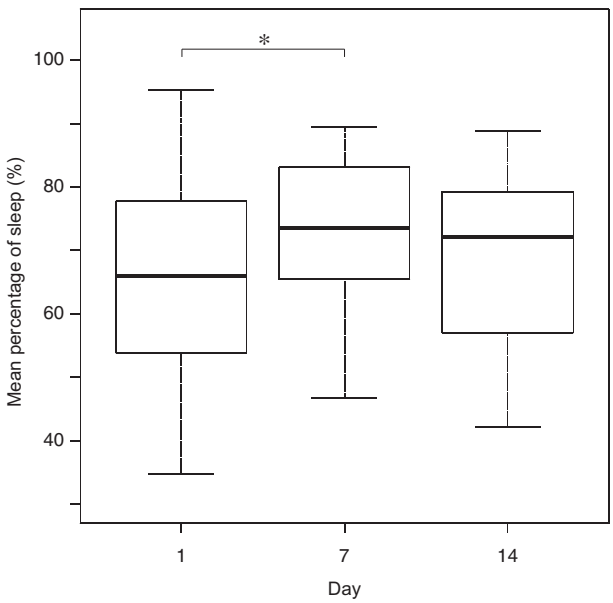

FIGURE 7. Development of the sleep efficiency of preterm infants over the study period, the sleep efficiency is the registered sleep duration divided by the total time of the recorded activity frequencies for every infant. *$p < 0.05$.

weeks of life, whereas other authors observed significantly higher cortisol concentrations only up to the first 5–7 days of life (Doerr et al., 1988; Economou et al., 1993; Scott & Watterberg, 1995). Economou et al. (1993) measured the cortisol levels of the same infants in 6-h intervals for 30 days and compared preterm with term infants, while in other studies, the cortisol concentration was only measured once or twice a day and was observed for different premature infants at varying ages (Doerr et al., 1988; Kidd et al., 2005; Scott & Watterberg, 1995; Vermes et al., 1980). The comparison between sick and healthy preterm babies indicated more pronounced cortisol values in sick babies after the third day of life and the highest cortisol concentrations in the evening. In contrast, healthy preterm infants showed significantly lower mean cortisol values at 20:00 h than at 08:00 or 14:00 h (Economou et al., 1993). Economou et al. (1993) related the trend towards higher values in the evening in sick preterm infants to medical practices that they undergo during the day. In general, Economou et al. (1993) observed higher cortisol concentrations in preterm infants than in full-term babies. Labor stress during vaginal or caesarean delivery has been reported to increase the cortisol value of neonates only up to the first 18–24 h of life (Klug et al., 2000; Pohjavuori et al., 1985). An early separation of the premature infant from her/his mother, continuous light and noise in the neonatal care unit as well as regular medical and nursing procedures in the first days of life could be further stress factors for a premature infant which increase cortisol values compared to full-term neonates (Newnham et al., 2009).

The pre-term neonates observed in this study showed individually different ultradian activity rhythm with a predominant period length of 4 h during the first 3 weeks of life. Previous studies reported similar results with ultradian frequencies in the activity-rest patterns of preterm infants during the first week of life (Glotzbach et al., 1995; Korte et al., 2001; Mirmiran & Kok, 1991; Nishihara et al., 2002). The appearance of circadian activity rhythm was represented in some studies at a mean age of eight postnatal weeks in preterm and term babies with a parallel development of the cortisol circadian rhythm (Antonini et al., 2000; Castro et al., 2000; McMillen et al., 1991; Shimada et al., 1993). Hellbrügge et al. (1956) described the first appearance in the heart rate rhythm within the second month of life, more obvious in term than in preterm infants. Glotzbach et al. (1995) documented pronounced ultradian rhythms in activity, heart rate and skin temperature with a period length of 3–4 h which were strongly related to feeding and caregiving procedures. The delayed emergence of circadian rhythm could also be a ''masking'' effect which is disrupted by medical and caregiving interventions as well as arrhythmic lightning in many neonatal units. It is well documented that light has a direct effect on the human circadian oscillator (Czeisler et al., 1986) and cycled light may stabilize circadian rhythms in adults as well as in preterm infants on NICU (Honma et al., 1987). Therefore, it appears to be very important to create a day–night rhythm on NICU as it might supports the synchronization of neonates' behavior and hormonal rhythms to environmental Zeitgebers (McMillen et al., 1991; Mirmiran & Ariagno, 2000). In full-term neonates, Korte et al. (2001) and Wulff & Siegmund (2000) found already a circadian

frequency during the first 2 weeks with actigraphic recordings.

Further analysis with the ActiLife Score indicated that preterm infants mainly show quiet activity bouts during the first days of life. However, the low activity bouts increased in the second and third weeks of the study period and the middle and high activity frequencies decreased over time. Moreover, the average sleep time duration increased 6.6% from the first study day to the 17th study day and 3.2% from the 1st to the 14th day. These results agree with previous studies which reported an increase in the duration of quiescence bouts and quiet sleep of preterm infants throughout the first weeks of life (Cioni & Prechtl, 1990; Giganti et al., 2001a,b, 2006). Furthermore, Korte et al. (2001) hypothesized that the increase in average nightly sleep time indicates the beginning of diurnal wake–sleep patterns. In full-term neonates the mean duration of night sleep time compared to their daily average sleep time was shown to be prolonged earlier than in preterm neonates. These results could therefore indicate an earlier beginning of a day–night differentiation in full-term infants (Ardura et al., 1995; Korte et al., 2001), possibly due to a faster development of the circadian clock mechanisms.

Additionally, in this study, the infants showed the highest peak of activity during the night-time in the first days of life which gradually reduced in the following weeks. The study of Giganti et al. (2001a,b) showed by video recording that preterm infants have the highest presence of wakefulness in the night between 02:00 and 05:00 h. In contrast, full-term infants showed the same patterns in the afternoon and evening between 17:00 and 20:00 h (Giganti et al., 2001a,b; Glotzbach et al., 1995). Compared with the literature our results on activity patterns of preterm neonates are more similar to the recorded activity behavior of fetuses which also show a greater amount of motility in the night-time than in the daytime (de Vries et al., 1987; Patrick et al., 1982; Roberts et al., 1979). In addition, long-term investigations revealed that the activity–rest behavior between preterm and full-term infants was still different at the age of 12 and 20 months. The night sleep quality of preterm infants was less restful and the sleep duration was partly reduced compared to full-term infants (Asaka & Takada, 2010; Gossel-Symank et al., 2004). A similar reduced sleep quality could also be found in infants with attention deficit/hyperactivity disorder (ADHD; Dagan et al., 1997; Gossel-Symank et al., 2004). Further, studies reported that very low birth weight children had an increased risk to suffer of ADHD (Botting et al., 1997). Therefore, actigraphic measurements might be very useful for an earlier screening and assessment of sleep disorders of preterm infants. As a matter of fact, sleep patterns in the first week of life could be predictive for the occurrence of sleep disorders in preterm infants during the first 3 years of life (Glotzbach et al., 1995; Whitney & Thoman, 1993).

Effect of acoustic stimulation on the activity behavior and cortisol concentration

In the present study, there were no statistically significant differences on the preterm infants' activity–rest behavior and wake–sleep patterns after auditive stimulation (e.g. audiotaped music or maternal voice). Also, Chapman (1978) found no effect in long-term motor activity of preterm infants who were stimulated by lullaby and taped maternal voice. Nevertheless, that study was limited by a small sample size, the data were collected at three different hospitals and the age of the preterm infants ranged widely from 26 to 33 of gestational weeks. Furthermore, a recent study of Loewy et al. (2013) revealed a significant increase in positive sleep patterns of preterm neonates who were stimulated with a timbre instrument which simulates a heartbeat sound that is similar of the neonates' environment in the womb. Lullabies which were sung live to the infants by their parents decreased the activity level especially after the acoustic intervention, but had less influence on the long-term sleep quality (Arnon et al., 2006; Loewy et al., 2013). In addition, we observed no significant decrease of the cortisol concentration neither after the stimulation with lullabies nor after the maternal voice stimulus.

A positive impact of music therapy on cortisol concentrations has only been reported on adults and children and had, until the present study, never been studied in preterm infants. Patients (<18 years) who listened to soft and relaxing music on the first postoperative day or at the intensive care unit showed significantly lower cortisol levels compared to the control group (Nilsson, 2009; Trappe, 2012).

Limitations of this study

It is possible that in the present study we could not demonstrate an influence of acoustic stimulation on the cortisol levels of preterm infants as we might have not captured the change in cortisol levels. Obtaining more samples at different time points after the interventions would have been helpful, but to obtain consensus from the parents of the preterm infants would have been nearly impossible. Furthermore, the analysis of cortisol data was limited by the loss of insufficient saliva samples as some children were discharged before the end of the study, some infants had a very dry mucosa due to phototherapy or drugs and finally, some samples were lost due to the evaporation of saliva after storing. In addition, we considered cortisol as a marker of stress response of preterm infants to medical interventions, light, noise and caregiving procedures on a neonatal unit, but no direct stress stimulus was given before the music intervention (e.g. blood sampling). Regarding the activity–rest analysis, there are also limitations concerning the sleep–wake pattern of preterm infants, since a validated algorithm for preterm infants has not been developed until now. Nevertheless, recent studies gave evidence that data monitored by actigraphs highly correlate with data from sleep diaries (Sadeh, 1996,

2011). Finally, our results are limited to late gestation premature babies as our infants were born at a gestational age of 30 weeks and more.

CONCLUSION

We could not detect a circadian or an ultradian rhythm in salivary cortisol of premature infants in the first weeks of life. Also, no significant impact of the acoustic stimulation on the development of activity–rest patterns or the cortisol concentration of premature neonates was observed. The majority of preterm neonates showed an ultradian rhythm of their activity behavior with a prominent period length of 4 h in the first 3 weeks of life. Activity and salivary cortisol levels of the neonates were significantly higher during night-time during the first week of life. The activity–rest behavior of the preterm changed during the study period, with quiet activity bouts increasing and greater/faster activity movements decreasing within the first 3 weeks after birth. In addition, the sleep efficiency of the infants increased slightly during the study period. Thus preterm neonates begin to develop rhythmicity during the first 3 weeks of life; interestingly acoustic stimulation did not have a promoting effect.

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DECLARATION OF INTEREST

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REFERENCES

Ancoli-Israel S, Cole R, Alessi C, et al. (2003). The role of actigraphy in the study of sleep and circadian rhythms. Sleep 26:342–92.

Antonini S, Jorge S, Moreira A. (2000). The emergence of salivary cortisol circadian rhythm and its relationship to sleep activity in preterm infants. Clin Endocrinol 52:423–6.

Antonucci R, Porcella A, Fanos V. (2009). The infant incubator in the neonatal intensive care unit: Unresolved issues and future developments. J Perinat Med 37:587–98.

Ardura J, Andres J, Aldana J, Revilla M. (1995). Development of sleep-wakefulness rhythm in premature babies. Acta Paediatr 84:484–9.

Arnon S, Shapsa A, Forman L, et al. (2006). Live music is beneficial to preterm infants in the neonatal intensive care unit environment. Birth 33:131–6.

Asaka Y, Takada S. (2010). Activity-based assessment of the sleep behaviors of VLBW preterm infants and full-term infants at around 12 months of age. Brain Dev 32:150–5.

Aschoff J. (1954). Zeitgeber der tierischen Tagesperiodik. Naturwissenschaften 4:49–56.

Aschoff J, Wever R. (1976). Human circadian rhythms: A multi-oscillatory system. Fed Proc 35:236–2.

Bates D, Maechler M, Bolker B, Walker S. (2014). lme4: Linear mixed-effects models using Eigen and S4. Available from: http://CRAN.R-project.org/package=lme4 [last accessed 11 Aug 2014].

Battin M, Bevan C, Harding J. (2007). Repeat doses of antenatal steroids and hypothalamic-pituitary-adrenal axis (HPA) function. Am J Obstet Gynecol 197:40.e1–6.

Behrends J, Bischofberger J, Deutzmann R. (2012). Duale Reihe Physiologie [Physiology of Duale Reihe]. Stuttgart: Thieme.

Bettendorf M, Albers N, Bauer J, et al. (1998). Longitudinal evaluation of salivary cortisol levels in full-term and preterm neonates. Horm Res 50:303–8.

Botting N, Powls A, Cooke R, Marlow N. (1997). Attention deficit hyperactivity disorders and other psychiatric outcomes in very low birthweight children at 12 years. J Child Psychol Psychiatry 38:931–41.

Boyle E, Poulsen G, Field D, et al. (2012). Effects of gestational age at birth on health outcomes at 3 and 5 years of age: Population based cohort study. BMJ 344:896.

Caine J. (1991). The effects of music on the selected stress behaviors, weight, caloric and formula intake, and length of hospital stay of premature and low birth weight neonates in a newborn intensive care unit. J Music Ther 28:180–92.

Calixto C, Martinez F, Jorge S, et al. (2002). Correlation between plasma and salivary cortisol levels in preterm infants. J Pediatr 140:116–18.

Caskey M, Stephens B, Tucker R, Vohr B. (2011). Importance of parent talk on the development of preterm infant vocalizations. Pediatrics 128:910–16.

Castro M, Elias P, Martinelli Jr C, et al. (2000). Salivary cortisol as a tool for physiological studies and diagnostic strategies. Braz J Med Biol Res 33:1171–5.

Chapman J. (1978). The relationship between auditory stimulation and gross motor activity of short-gestation infants. Res Nurs Health 1:29–36.

Chou I, Lien H, Lin H, et al. (2011). The relationship of salivary and cord blood cortisol in preterm infants. J Pediatr Endocrinol Metab 24:85–8.

Cioni G, Prechtl H. (1990). Preterm and early postterm motor behaviour in low-risk premature infants. Early Hum Dev 23: 159–91.

Czeisler C, Allan J, Strogatz S, et al. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science 233:667–71.

Dagan Y, Zeevi-Luria S, Sever Y, et al. (1997). Sleep quality in children with attention deficit hyperactivity disorder: An actigraphic study. Psychiatry Clin Neurosci 51:383–6.

de Vries J, Visser G, Mulder E, Prechtl H. (1987). Diurnal and other variations in fetal movement and heart rate patterns at 20–22 weeks. Early Hum Dev 15:333–48.

de Weerth C, Zijl R, Buitelaar J. (2003). Development of cortisol circadian rhythm in infancy. Early Hum Dev 73:39–52.

Doerr H, Sippell W, Versmold H, et al. (1988). Plasma mineralocorticoids, glucocorticoids, and progestins in premature infants: Longitudinal study during the first week of life. Pediatr Res 23:525–9.

Economou G, Andronikou S, Challa A, et al. (1993). Cortisol secretion in stressed babies during the neonatal period. Horm Res 40:217–21.

Giganti F, Cioni G, Biagioni E, et al. (2001a). Activity patterns assessed throughout 24-hour recordings in preterm and near term infants. Dev Psychobiol 38:133–42.

Giganti F, Fagioli I, Ficca G, et al. (2001b). Preterm infants prefer to be awake at night. Neurosci Lett 312:55–7.

Giganti F, Ficca G, Cioni G, Salzarulo P. (2006). Spontaneous awakenings in preterm and term infants assessed throughout 24-h video-recordings. Early Hum Dev 82:435–40.

Glotzbach S, Edgar D, Ariagno R. (1995). Biological rhythmicity in preterm infants prior to discharge from neonatal intensive care. Pediatrics 95:231–7.

Gossel-Symank R, Grimmer I, Korte J, Siegmund R. (2004). Actigraphic monitoring of the activity-rest behavior of preterm and full-term infants at 20 months of age. Chronobiol Int 21: 661–71.

Halberg F. (1963). Circadian (about twenty-four-hour) rhythms in experimental medicine. Proc R Soc Med 56:253–7.

Hellbrügge T, Lange J, Rutenfranz J. (1956). Development of diurnal periodical pulse rate changes in children. Z Kinderheilkd 78:703–22.

Herrington C, Olomu I, Geller S. (2004). Salivary cortisol as indicators of pain in preterm infants: A pilot study. Clin Nurs Res 13:53–68.

Honma K, Honma S, Wada T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. Experientia 43:572–4.

Iwata O, Okamura H, Saitsu H, et al. (2013). Diurnal cortisol changes in newborn infants suggesting entrainment of peripheral circadian clock in utero and at birth. J Clin Endocrinol Metab 98:E25–32.

Kari M, Raivio K, Stenman U, Voutilainen R. (1996). Serum cortisol, dehydroepiandrosterone sulfate, and steroid-binding globulins in preterm neonates: Effect of gestational age and dexamethasone therapy. Pediatr Res 40:319–24.

Kaufmann J, Kirchschlager A, Moll K, et al. (2009). Exklusive Wiegenlieder CD-Sammlung, Vol. 1 [German Lullabies]. LE-Echterdingen: Carus-Verlag GmbH.

Kennaway D, Stamp G, Goble F. (1992). Development of melatonin production in infants and the impact of prematurity. J Clin Endocrinol Metab 75:367–9.

Kidd S, Midgley P, Nicol M, et al. (2005). Lack of adult-type salivary cortisol circadian rhythm in hospitalized preterm infants. Horm Res 64:20–7.

Klug I, Dressendorfer R, Strasburger C, et al. (2000). Cortisol and 17-hydroxyprogesterone levels in saliva of healthy neonates: Normative data and relation to body mass index, arterial cord blood ph and time of sampling after birth. Biol Neonate 78: 22–6.

Korte J, Wulff K, Oppe C, Siegmund R. (2001). Ultradian and circadian activity-rest rhythms of preterm neonates compared to full-term neonates using actigraphic monitoring. Chronobiol Int 18:697–708.

Krieger D, Allen W, Rizzo F, Krieger H. (1971). Characterization of the normal temporal pattern of plasma corticosteroid levels. J Clin Endocrinol Metab 32:266–84.

Krueger C. (2010). Exposure to maternal voice in preterm infants: A review. Adv Neonatal Care 10:13–18; quiz 19–20.

Lai T, Shih M, Wong S. (2006). A new approach to modeling covariate effects and individualization in population pharmacokinetics-pharmacodynamics. J Pharmacokinet Pharmacodyn 33:49–74.

Lemmer B. (2006). The importance of circadian rhythms on drug response in hypertension and coronary heart disease – from mice and man. Pharmacol Ther 111:629–51.

Lemmer B. (2008). Effects of music composed by Mozart and Ligeti on blood pressure and heart rate circadian rhythms in normotensive and hypertensive rats. Chronobiol Int 25: 971–86.

Loewy J, Stewart K, Dassler A, et al. (2013). The effects of music therapy on vital signs, feeding, and sleep in premature infants. Pediatrics 131:902–18.

Maier R, Obladen M. (2011). Neugeborenenintensivmedizin [Intensive care of preterm infants]. Berlin: Springer Verlag.

McMillen I, Kok J, Adamson T, et al. (1991). Development of circadian sleep-wake rhythms in preterm and full-term infants. Pediatr Res 29:381–4.

Mendez N, Abarzua-Catalan L, Vilches N, et al. (2012). Timed maternal melatonin treatment reverses circadian disruption of the fetal adrenal clock imposed by exposure to constant light. PLoS One 7:e42713.

Metzger D, Wright N, Veldhuis J, et al. (1993). Characterization of pulsatile secretion and clearance of plasma cortisol in premature and term neonates using deconvolution analysis. J Clin Endocrinol Metab 77:458–63.

Mirmiran M, Ariagno R. (2000). Influence of light in the NICU on the development of circadian rhythms in preterm infants. Chronobiol Newborn Period 24: 247–57.

Mirmiran M, Kok J. (1991). Circadian rhythms in early human development. Early Hum Dev 26:121–8.

Moore R. (1973). Retinohypothalamic projection in mammals: A comparative study. Brain Res 49:403–9.

Moore R, Eichler V. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42:201–6.

Moore-Ede M, Czeisler C, Richardson G. (1983). Circadian timekeeping in health and disease: I. Basic properties of circadian pacemakers. N Engl J Med 309:469–76.

Neu M, Goldstein M, Gao D, Laudenslager M. (2007). Salivary cortisol in preterm infants: Validation of a simple method for collecting saliva for cortisol determination. Early Hum Dev 83: 47–54.

Newnham C, Inder T, Milgrom J. (2009). Measuring preterm cumulative stressors within the NICU: The neonatal infant stressor scale. Early Hum Dev 85:549–55.

Nilsson U. (2009). The effect of music intervention in stress response to cardiac surgery in a randomized clinical trial. Heart Lung 38:201–7.

Nishihara K, Horiuchi S, Eto H, Uchida S. (2002). The development of infants' circadian rest-activity rhythm and mothers' rhythm. Physiol Behav 77:91–8.

Patrick J, Campbell K, Carmichael L, et al. (1982). Patterns of gross fetal body movements over 24-hour observation intervals during the last 10 weeks of pregnancy. Am J Obstet Gynecol 142:363–71.

Philbin M, Klaas P. (2000). Evaluating studies of the behavioral effects of sound on newborns. J Perinatol 20:S61–7.

Pinheiro J, Bates D, DebRoy S, et al. (2014). nlme: Linear and nonlinear mixed effects models. Available from: http://CRAN.R-project.org/package=nlme [last accessed 11 Aug 2014].

Pohjavuori M, Rovamo L, Laatikainen T. (1985). Plasma immunoreactive beta-endorphin and cortisol in the newborn infant after elective caesarean section and after spontaneous labour. Eur J Obstet Gynecol Reprod Biol 19:67–74.

Potts A, Cheeseman J, Warman G. (2011). Circadian rhythms and their development in children: Implications for pharmacokinetics and pharmacodynamics in anesthesia. Paediatr Anaesth 21:238–46.

Price D, Close G, Fielding B. (1983). Age of appearance of circadian rhythm in salivary cortisol values in infancy. Arch Dis Child 58: 454–6.

R Development Core Team. (2008). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for

Statistical Computing. Available from: http://www.R-project.org [last accessed 11 Aug 2014].
Redfern P, Lemmer B. (1997). Physiology and pharmacology of biological rhythms. Berlin: Springer Verlag.
Reppert S, Schwartz W. (1983). Maternal coordination of the fetal biological clock in utero. Science 220:969–71.
Rivkees S, Mayes L, Jacobs H, Gross I. (2004). Rest-activity patterns of premature infants are regulated by cycled lighting. Pediatrics 113:833–9.
Roberts A, Little D, Cooper D, Campbell S. (1979). Normal patterns of fetal activity in the third trimester. Br J Obstet Gynaecol 86: 4–9.
Sadeh A. (1996). Evaluating night wakings in sleep-disturbed infants: A methodological study of parental reports and actigraphy. Sleep 19:757–62.
Sadeh A. (2011). The role and validity of actigraphy in sleep medicine: An update. Sleep Med Rev 15:259–67.
Sadeh A, Acebo C. (2002). The role of actigraphy in sleep medicine. Sleep Med Rev 6:113–24.
Saint Exupéry de A. (1995). Der kleine Prinz [The little prince]. Düsseldorf: Karl-Rauch Verlag.
Santiago L, Jorge S, Moreira A. (1996). Longitudinal evaluation of the development of salivary cortisol circadian rhythm in infancy. Clin Endocrinol (Oxf) 44:157–61.
Scott S, Watterberg K. (1995). Effect of gestational age, postnatal age, and illness on plasma cortisol concentrations in premature infants. Pediatr Res 37:112–16.
Seron-Ferre M, Ducsay C, Valenzuela G. (1993). Circadian rhythms during pregnancy. Endocr Rev 14:594–609.
Seron-Ferre M, Torres-Farfan C, Forcelledo M, Valenzuela G. (2001). The development of circadian rhythms in the fetus and neonate. Semin Perinatol 25:363–70.
Shimada M, Segawa M, Higurashi M, Akamatsu H. (1993). Development of the sleep and wakefulness rhythm in preterm infants discharged from a neonatal care unit. Pediatr Res 33: 159–63.
Snow G. (2006). The blockrand Package: Randomization for block random clinical trials. Salt Lake City, Utah. Available from: http://CRAN.R-project.org/package=blockrand [last accessed 11 Aug 2014].
So K, Buckley P, Adamson T, Horne R. (2005). Actigraphy correctly predicts sleep behavior in infants who are younger than six months, when compared with polysomnography. Pediatr Res 58:761–5.
Standley J. (2002). A meta-analysis of the efficacy of music therapy for premature infants. J Pediatr Nurs 17:107–13.
Standley J, Moore R. (1995). Therapeutic effects of music and mother's voice on premature infants. Pediatr Nurs 21: 509–12, 574.
Stephan F, Zucker I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci USA 69:1583–6.
Tollenaar M, Jansen J, Beijers R, et al. (2010). Cortisol in the first year of life: Normative values and intra-individual variability. Early Hum Dev 86:13–16.
Trappe H. (2012). Role of music in intensive care medicine. Int J Crit Illn Inj Sci 2:27–31.
Tsai S, Thomas K, Lentz M, Barnard K. (2012). Light is beneficial for infant circadian entrainment: An actigraphic study. J Adv Nurs 68:1738–47.
van Cauter E, Leproult R, Kupfer D. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab 81:2468–73.
Vermes I, Dohanics J, Toth G, Pongracz J. (1980). Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. Horm Res 12:237–44.
Whitney M, Thoman E. (1993). Early sleep patterns of premature infants are differentially related to later developmental disabilities. J Dev Behav Pediatr 14:71–80.
Wulff K, Siegmund R. (2000). Circadian and ultradian time patterns in human behaviour: Part 1: Activity monitoring of families from prepartum to postpartum. Biol Rhythm Res 31:581–602.
Zuther P, Gorbey S, Lemmer B. (2009). Chronos-Fit 1.06. Heidelberg. Available from: http://www.ma.uni-heidelberg.de/inst/phar/lehre/chrono.html [last accessed 11 Aug 2014].