scientific reports

Check for updates

OPEN

Neural conduction impairment of the auditory brainstem in babies with bronchopulmonary dysplasia while being diagnosed

Ze Dong Jiang[1,2]✉, Rong Yin[1,2], James Ken Jiang[1] & Cui Wang[1]

Brainstem neural conduction was investigated in 78 very low birthweight (VLBW) at 36 weeks of postconceptional age while diagnosing neonatal bronchopulmonary dysplasia (BPD). The maximum length sequence technique was used to study brainstem auditory evoked response (MLS BAER). Compared with healthy controls, VLBW babies diagnosed with BPD manifested significant prolongation of wave V latency and I-V, I-III and III-V intervals at all 91–910/s clicks. Those diagnosed without BPD manifested only moderate prolongation of I-V and III-V intervals at higher click rates. There were marked differences in MLS BAER between babies with BPD and those without BPD. The babies with BPD manifested significantly longer wave V latency and I-V, I-III, and III-V intervals than those without BPD, particularly at higher click rates. Click rate-dependent changes in the I-V and III-V intervals were significantly increased in BPD babies. These results suggest that while diagnosing BPD the VLBW babies diagnosed with BPD have major brainstem neural impairment, whereas those without BPD have only minor impairment. The major MLS BAER abnormalities in BPD may offer an early biomarker of brainstem neural impairment for BPD.

Keywords Bronchopulmonary dysplasia, Brainstem impairment, Evoked potentials, Neural conduction, Perinatal brain injury

Babies born with a very low birthweight (VLBW) are prone to neonatal bronchopulmonary dysplasia (BPD), the most common pulmonary morbidity in VLBW babies[1–3]. Neonatal BPD constitutes a great risk of neonatal and post-neonatal mortality and a host of associated medical and developmental sequelae[4–9]. Although the long-term neurological outcome of neonatal BPD has been widely studied, little information is available on the neuropathology of the immature brain shortly after the development of neonatal BPD. The first study on the functional integrity of the auditory brainstem in VLBW babies with neonatal BPD revealed major impairment in the auditory brainstem when these babies were at term age[10]. Nevertheless, it remains unclear if there is any auditory brainstem impairment occurring before term age in VLBW babies with BPD. The understanding is of great importance for early detection of neurological abnormalities and future study of early neuroprotective measures for VLBW babies with BPD before term age.

Diagnosing BPD is usually made at PCA 36 weeks, i.e., shortly before term or near-term[11,12]. It is likely that the brainstem auditory impairment already exists by then. We hypothesized that the immature auditory brainstem in BPD babies is already adversely affected by the development of BPD at the time when they were being diagnosed with BPD. Thus, we investigated the functional integrity of the auditory brainstem in VLBW babies at PCA 36 weeks while diagnosing BPD. Our first aim was to explore if the auditory brainstem is impaired before term in BPD babies, improving our understanding of the neuropathology of the immature auditory brainstem at the time when BPD is being diagnosed and contributing to early detection of neurological impairment before term. The second aim was to differentiate differences in the functional integrity of the auditory brainstem between VLBW babies with BPD and those without BPD (non-BPD). These aims were achieved by recording and analysing the maximum length sequence brainstem auditory evoked response (MLS BAER) — a relatively new technique to improve the detection of neuropathology that involves the auditory brainstem[10,13–15]. Particular attention was paid to assessing brainstem neural conduction, reflected by MLS BAER latencies and especially interpeak intervals[13,14,16].

[1]Division of Neonatology, Children's Hospital of Fudan University, 399 Wan Yuan Road, Shanghai 201102, China. [2]Ze Dong Jiang and Rong Yin contributed equally to this work. ✉email: jiangzedong-oxshang@hotmail.com

Methods

Design and study participants

Seventy-eight VLBW babies were recruited at 36 weeks of PCA when the diagnosis of BPD was carried out at the neonatal unit of the Hospital. They were born with a birth weight ≤ 1,500 g and a gestation ranging between 24 and 31 weeks. Following a detailed explanation of the study, informed consent was obtained from the parents of each baby. The developing auditory brainstem can be directly or indirectly affected by a range of perinatal complications or problems, such as hypoxia-ischemia, necrotizing enterocolitis, hyperbilirubinemia at a serum level requiring exchange transfusion, congenital malformation of the CNS, and bacterial meningitis[14,15]. Those babies who had these problems had been excluded from study entry to minimize any major confounding effects. All passed the neonatal hearing screening program with otoacoustic emission. The healthy control group was comprised of 34 near-term babies who did not have any aforementioned major perinatal complications or problems (20 boys and 14 girls). These babies were born at 35–36 weeks of gestation and with a birthweight greater than 1,500 g (Table 1). They also all passed the neonatal hearing screening program with otoacoustic emission.

As previously reported, the diagnostic criteria for BPD include a requirement for supplementary oxygen or ventilatory support beyond 36 weeks of PCA to maintain $PaO_2 > 50$ mmHg, clinical signs of chronic lung respiratory disease and radiographic evidence of BPD (persistent strands of density in both lungs)[10]. Of the 78 VLBW babies, 37 were diagnosed with BPD (21 boys and 16 girls), and the remaining 41 were without BPD (non-BPD, 23 boys and 18 girls). The number of babies in each group was greater than the required 16 babies to achieve statistical significance for comparing different groups in MLS BAER, which allowed to minimize any bias arising possible variation in individual babies and analysis data more reliably[16]. Of the BPD babies, 12 had grade 1 BPD, 17 had grade 2 BPD, and 8 had grade 3 BPD. A small number of babies were associated with intraventricular hemorrhages (IVH) and periventricular leucomalacia (PVL), diagnosed by cranial ultrasound scan. In the BPD group, 4 babies were associated with IVH, and 6 with PVL (1 combined with IVH). In the non-BPD group, 3 babies were associated with IVH and 4 with PVL.

The main demographics of these babies are summarized in Table 1. There were no significant differences in birthweight and occipitofrontal head circumference at birth between the BPD and non-BPD groups. Gestation and head circumference at the time of testing in the BPD group were smaller than in the non-BPD group (Table 1). In the healthy control group, gestation, birthweight, and head circumference at birth were significantly greater than in those in the BPD and non-BPD groups (Table 1). The head circumference at testing was similar to that in the non-BPD group, which was greater than that in the BPD group (Table 1). However, the PCA, at which the diagnosis of BPD was made and MLS BAER was recorded blind to the results of the diagnosis, was almost identical for the two groups.

MLS BAER recording and analysis

The procedures performed in the present study involving human participants were approved by the Ethics Committee of the Institute, in accordance with the 1964 Helsinki Declaration and its later amendments. The protocols for recording MLS BAER were generally the same as previously reported[10,13,14]. All babies were tested at PCA 36 weeks in a quiet room in the neonatal unit using a Spirit 2000 Evoked Potential System (Nicolet Biomedical Inc. Madison, WI, USA). Subject to the baby's clinical conditions, some babies were re-tested once or twice to obtain reliable recordings on the same day.

Very briefly, three gold-plated disk electrodes were placed at the middle forehead (positive), the ipsilateral earlobe (negative), and the contralateral earlobe (ground), respectively. The acoustic stimuli were rarefaction

BAER measures	Healthy	Non-BPD	BPD	F	*P*
Gestation (weeks)					
Mean ± SD	35.9 ± 0.5	28.7 ± 1.1***	28.0 ± 1.3***§§		< 0.001
Range	35–36	26–31	26–30	475.3	
PCA (weeks)					
Mean ± SD	36.5 ± 0.4	36.5 ± 0.4	36.6 ± 0.3	0.3	0.720
Birthweight (g)					
Mean ± SD	2268 ± 665	1220 ± 180***	1147 ± 164***		< 0.001
Range	1,515–3216	698– 1490	770–1475	88.0	
HC at birth (ms)					
Mean ± SD	31.2 ± 3.0	26.5 ± 1.4*	26.3 ± 1.9**		< 0.001
Range	26.0–35.0	23.0-29.2	23.0–29.0	47.7	
HC at testing (ms)					
Mean ± SD	31.8 ± 2.5	32.0 ± 1.0	30.6 ± 2.0***§§§	9.3	< 0.001
Range	27.0–35.0	30.0–34.0	26.5–33.2		

Table 1. Main demographics of BPD, non-BPD and healthy control groups. HC refers to head circumference. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ is the significance for the comparison of non-BPD and BPD groups with Healthy group. $^{§§}P < 0.01$, $^{§§§}P < 0.001$ are the significance for the comparison between non-BPD and BPD groups.

clicks of 100 μs at 60 dB normal hearing level (nHL). The clicks were delivered to the left ear through a headphone at the repetition rates of 91–910/s in the first run and at the rates of 910–91/s in the reverse run. During the signal averaging, amplitude artifact rejection was active to eliminate any on-line signals with amplitude exceeding +/– 25 μV to minimize artefacts. The evoked brain responses to 1,500 trains of clicks were preamplified, bandpassed between 100 and 3,000 Hz, and then averaged for each run of recording. Two runs for each recording condition were made for reproducibility.

Analysis of MLS BAER waveforms was carried out by two independent evaluators without knowing the baby's clinical data and diagnosis. The peaks of MLS BAER waves I, III and V were identified in the well-formed MLS BAER waveforms. The latency of each wave component was measured, and interpeak intervals of I-V, I-III and III-V were then derived, as previously schematically shown[14,16]. The III-V/I-III interval ratio was also calculated. Measurements of these MLS BAER variables from two replicable recordings to each stimulus condition were averaged for further statistical analysis.

Statistical analysis

All MLS BAER wave latencies and interpeak intervals followed normal distribution in the one-sample Kolmogorov-Smirnov test. Analysis of variance (ANOVA) was used to compare the mean and standard deviation of each MLS BAER variable between different groups at each stimulus condition. A 2-tailed value of $p < 0.05$ was judged to be statistically significant. The statistical analyses were conducted using a version 29 SPSS package (Chicago, IL). First, the MLS BAER data obtained from the BPD, non-BPD, and healthy control groups were compared for any significant differences among the three groups of babies. Then, the MLS BAER data obtained in the BPD group were compared with those in the healthy control group to detect any abnormality in VLBW babies with BPD. The MLS BAER data in the non-BPD group were then compared with those in the healthy control group to detect any abnormalities in VLBW babies without BPD and assess the effects of very preterm birth and VLBW on the developing auditory brainstem. Finally, a comparison of MLS BAER data was made between the BPD and non-BPD groups to identify any differences in brainstem neural conduction between the two groups of babies at the time of diagnosing BPD and assess the effect of neonatal BPD on the developing auditory brainstem before term age.

Moreover, click rate-dependent changes in MLS BAER variables were examined by linear regression analysis between MLS BAER variables and the repetition rate of clicks. Regression analysis was then performed for those MLS BAER variables that were significantly correlated with click rate. With the increase in the click rate from 91/s to 455/s, MLS BAER wave latencies and interpeak intervals increased progressively, i.e., the latencies and intervals were changed linearly with varying click rates. As the rate was further increased to 910/s, MLS BAER wave latencies and intervals did not show any further notable increase or change, and the measurements of these MLS BAER variables at 910/s clicks were similar to those at 455/s. It appeared that MLS BAER wave latencies and intervals reached a plateau at 455/s clicks. Further increasing the click rate to 910/s did not significantly increase the wave latencies and intervals, i.e., these MLS BAER variables were no longer changed linearly with increasing click rate. This was seen in all the BPD, non-BPD, and healthy control groups, as well as in our previous MLS BAER studies[13,14,16]. As such, the linear regression analysis was conducted between 91 and 455/s, instead of between 91 and 910/s in all three groups of babies in order to more accurately assess the linear relationship of MLS BAER wave latencies and intervals with click rate. Having obtained the latency-, and interval-rate functions, the slopes (or regression coefficients) for these functions were then calculated. Any slopes that were significantly greater than zero at the 0.05 level or better were compared between the BPD, normal term, and non-BPD groups using a Student t-test to detect any significant differences between groups in the click rate-dependent changes, i.e., the changes in MLS BAER variables with varying click rate, and assess the efficacy of synaptic transmission in the auditory brainstem[10,13,14,16].

Results

The PCA at which MLS BAER was recorded was almost the same in our BPD, non-BPD, and healthy control groups (Table 1). The hearing levels (i.e. the dB above the threshold of each infant) were similar for the three groups of babies; 48.4 ± 4.4 dB nHL, 48.8 ± 4.7 dB nHL, and 49.2 ± 3.7 dB nHL, respectively, for the BPD, non-BPD and healthy control groups, without any statistical significance between any of the three groups. Therefore, the measurements of MLS BAER variables obtained were comparable between groups without the influence of any notable differences in hearing level.

Comparison among BPD, non-BPD, and healthy control groups

The latency of MLS BAER wave I tended to be shorter in the BPD group than in the non-BPD and healthy control groups, but the differences among the three groups of babies were not statistically significant at any click rate (Fig. 1). There were only small differences in wave III latency among the three groups (Fig. 2). Wave V latency in the BPD group was longer than in the healthy control and non-BPD groups (Fig. 3). The latency differed significantly among the three groups at all 91–910/s clicks ($p < 0.05$ at 91 and 227/s, and $p < 0.001$ at 455 and 910/s) (Fig. 3). The I-V interval was longer in the BPD group than in both the non-BPD and healthy control groups, and differed significantly among the three groups at all rates ($p < 0.01$–0.001, Fig. 4). The I-III interval was longer in the BPD group than in the non-BPD and healthy control groups, and differed significantly at 91–455/s clicks among the three groups ($p < 0.05$–0.01) (Fig. 5). Similarly, the III-V interval was prolonged in the BPD group, and differed significantly among the three groups at 227–910/s clicks ($p < 0.05$–0.001) (Fig. 6). The III-V/I-III interval ratio was similar in the three groups at 91 and 227/s, but was greater in the BPD and non-BPD groups than in the healthy control group at higher rates. The ratio differed significantly among the three groups at 455 and 910/s click ($p < 0.001$ and 0.001) (Fig. 7).

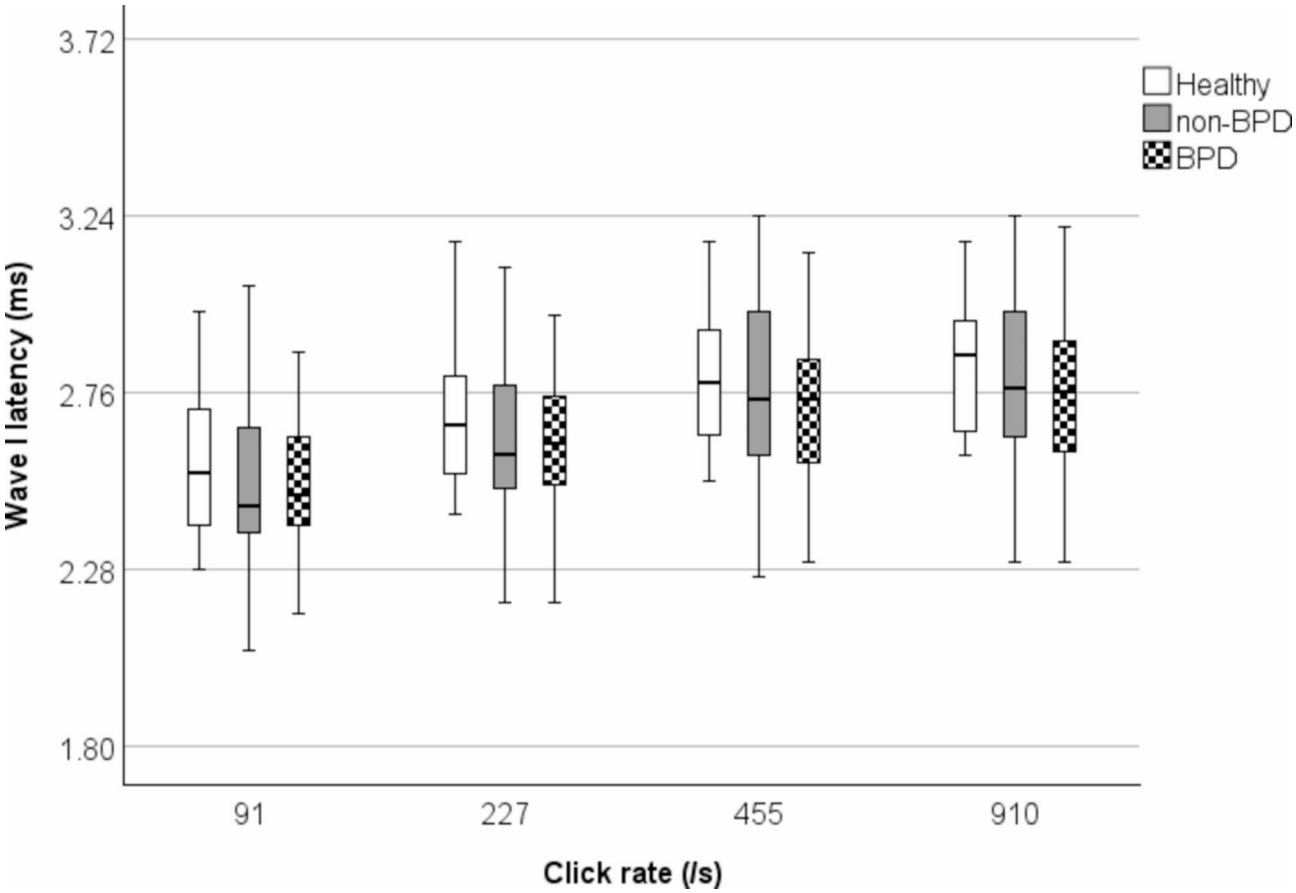

Fig. 1. Boxplot (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER wave I latency, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The latency shows small differences between different groups.

Comparison of BPD group with healthy control group

Clear differences were found between the two groups of babies in the MLS BAER variables that reflect brainstem neural conduction. Compared with the healthy control group, the BPD group demonstrated a decreased wave I latency at all 91–910/s clicks, but the difference was not statistically significant (Fig. 1). Wave III latency was similar in the two groups of babies (Fig. 2). Wave V latency in the BPD group was longer than in the healthy control group, particularly at higher click rates, and differed significantly at 455 and 910/s ($p < 0.001$ and 0.001) (Fig. 3). All interpeak intervals in the BPD group were significantly longer than in the healthy group. The I-V interval in the BPD group was significantly longer than in the healthy group at all click rates ($p < 0.05$–0.001) (Fig. 4). This was also the case of the III-V interval (all $p < 0.05$–0.001) (Fig. 6). The I-III interval in the BPD group was significantly longer than in the healthy group at 91, 227 and 910/s clicks (all $p < 0.05$) (Fig. 5). The III-V/I-III interval ratio in the BPD group was slightly greater than in the healthy control group at the lower rates of 91 and 227/s clicks, but significantly greater at higher rates of 455 and 910/s ($p < 0.01$ and 0.001) (Fig. 7).

Comparison of non-BPD group with healthy control group

The latencies of MLS BAER waves I and III were similar in the two groups at all click rates of 91–910/s (Figs. 1 and 2). Wave V latency in the non-BPD group was longer than in the healthy control group, which nearly reached statistical significance at the higher rates of 455 and 910/s (Fig. 3). The I-V and III-V intervals tended to be longer than in the healthy group. The differences were increased with increasing click rate, and reached statistical significance at 455/s clicks for the I-V interval ($p < 0.05$) and 455 and 910/s clicks for the III-V interval ($p < 0.01$ and 0.001) (Figs. 4 and 6). In contrast, the I-III interval in the non-BPD group was slightly shorter than in the healthy groups at higher click rates (Fig. 5). The III-V/I-III interval ratio in the non-BPD group was significantly greater than in the healthy group at 455 and 910/s clicks ($p < 0.01$ and 0.001) (Fig. 7).

Comparison of BPD group with non-BPD group

As presented above, the BPD group manifested major differences in MLS BAER wave latencies and interpeak intervals from the healthy control group, whereas the non-BPD group manifested only minor differences from the healthy group. The MLS BAER data were further directly compared between the BPD and non-BPD groups for any differences. There were small differences in wave I latency between the BPD and non-BPD groups at

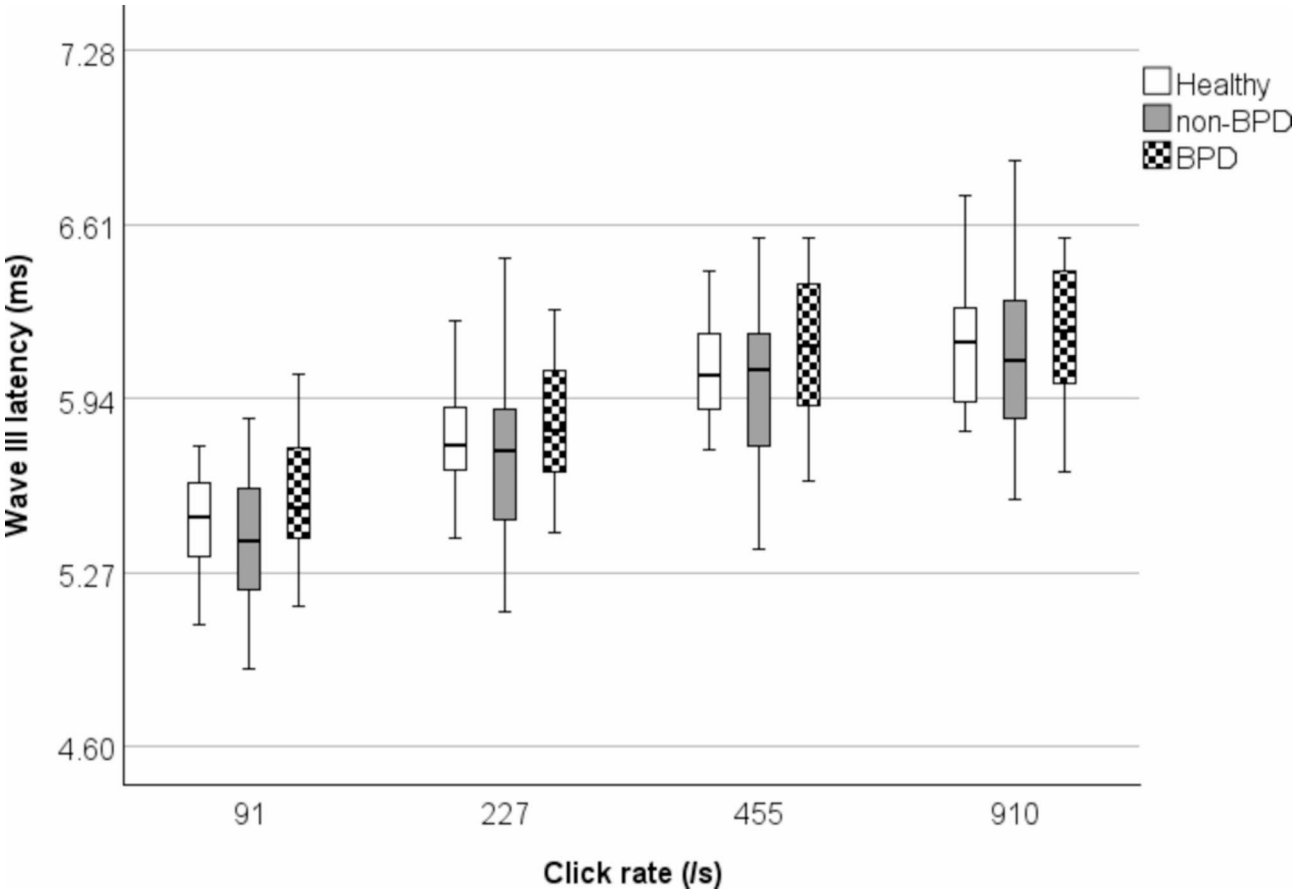

Fig. 2. Boxplot (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER wave III latency, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The latency shows small differences between different groups.

various click rates (Fig. 1). Wave III latency was slightly longer in the BPD group than in the non-BPD group, without any statistical significance at any click rate (Fig. 2). Nevertheless, wave V latency in the BPD group was significantly longer than in the non-BPD group at all 91–910/s clicks (all $p < 0.05$) (Fig. 3). The I-V interval in the BPD group was significantly longer than in the non-BPD group at all click rates particularly higher rates ($p < 0.05$–0.001) (Fig. 4). Of the two smaller intervals, the I-III in the BPD group was significantly longer than in the non-BPD group at all rates ($p < 0.05$–0.01) (Fig. 5), whereas the III-V interval was also longer than in the non-BPD group at all click rates, which differed significantly at higher rates of 455 and 910/s ($p < 0.01$ and 0.01) (Fig. 6). No significant difference was observed in the III-V/I-III interval ratio between the BPD and non-BPD groups.

Comparison of the changes in MLS BAER with varying click rates between different groups

The intercepts of latency-functions for MLS BAER waves I, III and V in the BPD group were generally slightly greater than in the healthy control and non-BPD groups (Table 2). This was also the case for the intercepts of I-V and III-V interval-rate functions (Table 3). These intercepts in the non-BPD group were slightly smaller than in the healthy control group.

As shown in Tables 2 and 3, the slopes of all latency- and interval-rate functions were significantly greater than zero at the 0.001 level, which was true for all three groups of babies. Therefore, comparisons of the slopes were made between these groups using Student's t-test to identify any differences in the changes in MLS BAER with varying click rates, i.e., the click rate-dependent changes. No obvious differences were seen for the slopes of wave I and III latency-rate functions between any groups. The BPD group had the greatest slope for wave V latency-rate function among the three groups, although no statistical significance was found in the differences in the slope between any groups.

The slope for the I-V interval-rate function was the greatest in the BPD group, which was significantly greater than in the healthy control group ($t = 2.012$, $p < 0.05$) and moderately greater than in the non-BPD group (Table 3). The slope in the non-BPD group was only slightly greater than in the healthy control group. The slope for the I-III interval-rate function was similar in the three groups of babies. However, the slope for the III-V interval-rate function in the BPD group was significantly greater than in the healthy control group ($t = 3.707$, $p < 0.01$) (Table 3), and greater than in the non-BPD group which nearly reached statistical significance. This

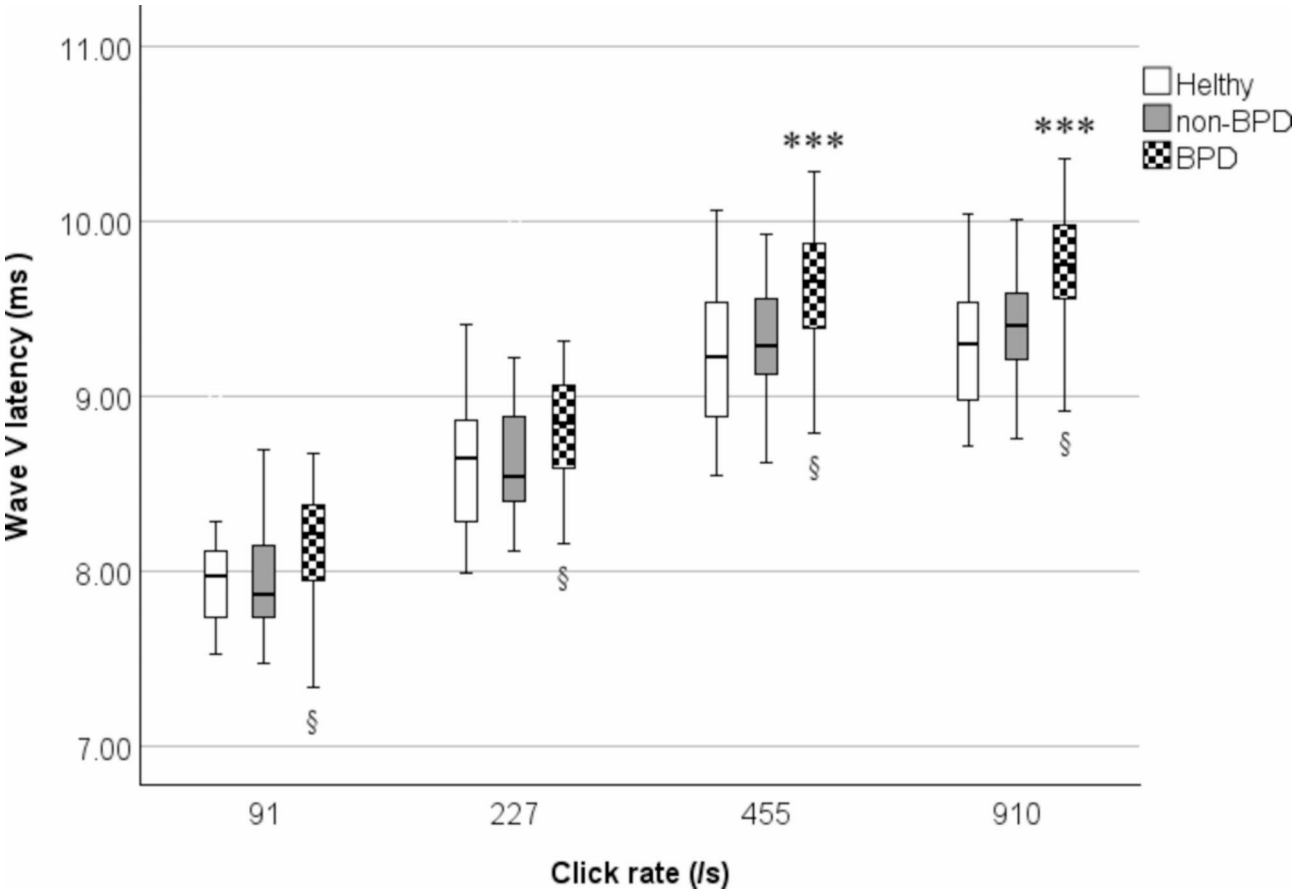

Fig. 3. Boxplot (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER wave V latency, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The latency in BPD group is significantly longer than in healthy and non-BPD groups at all click rates, and the differences increase with increasing click rate. $^{***}p < 0.001$ is the significance for the comparison of BPD group with healthy group; $^{§}p < 0.05$ is the significance for the comparison between BPD and non-BPD groups.

slope in the non-BPD group was also greater than in the healthy control group ($t = 2.146$, $p < 0.05$) (Table 3). The slope for the III-V/I-III interval ratio-rate function was the greatest in the BPD group, although the differences were not statistically significant between any groups.

Comparison of MLS BAER between different groups after exclusion of the babies with IVH and PVL

The MLS BAER data obtained from the babies who did not have IVH and PVL were generally similar to those when all babies were included, with only small variations. The differences between different groups in the MLS BAER results were also similar to those when all babies were included. Figures 8, 9, 10 and 11 show the measurements of major MLS BAER variables, reflecting neural conduction in the auditory brainstem, at various click rates in all groups when the babies with IVH and PVL were included and when they were excluded. In the BPD babies without IVH and PVL (BPDc group), wave V latency was significantly longer than in the non-BPD babies without IVH and PVL (non-BPDc group) across all 91–910/s clicks (all $p < 0.05$) (Fig. 8). The I-V interval in the BPDc group was significantly longer than in the non-BPDc group at higher rates of 227–910/s clicks ($p < 0.05$–0.01) (Fig. 9). The I-III interval in the BPDc group was significantly longer than in the non-BPDc group at all rates ($p < 0.05$–0.01) (Fig. 10), and the III-V interval was significantly longer at higher rates of 455 and 910/s ($p < 0.05$ and 0.05) (Fig. 11). No significant differences existed in any MLS BAER variables between the BPDc and BPD groups and between the non-BPDc and non-BPD groups at any click rates.

Discussion

Neural conduction impairment of the auditory brainstem in babies with BPD while being diagnosed

In comparison with healthy controls, VLBW babies who were diagnosed with BPD manifested a significant prolongation of wave V latency and I-V, I-III and III-V intervals. As the most important BAER variable for the functional integrity of the auditory brainstem, the I-V interval is referred to as brainstem conduction time,

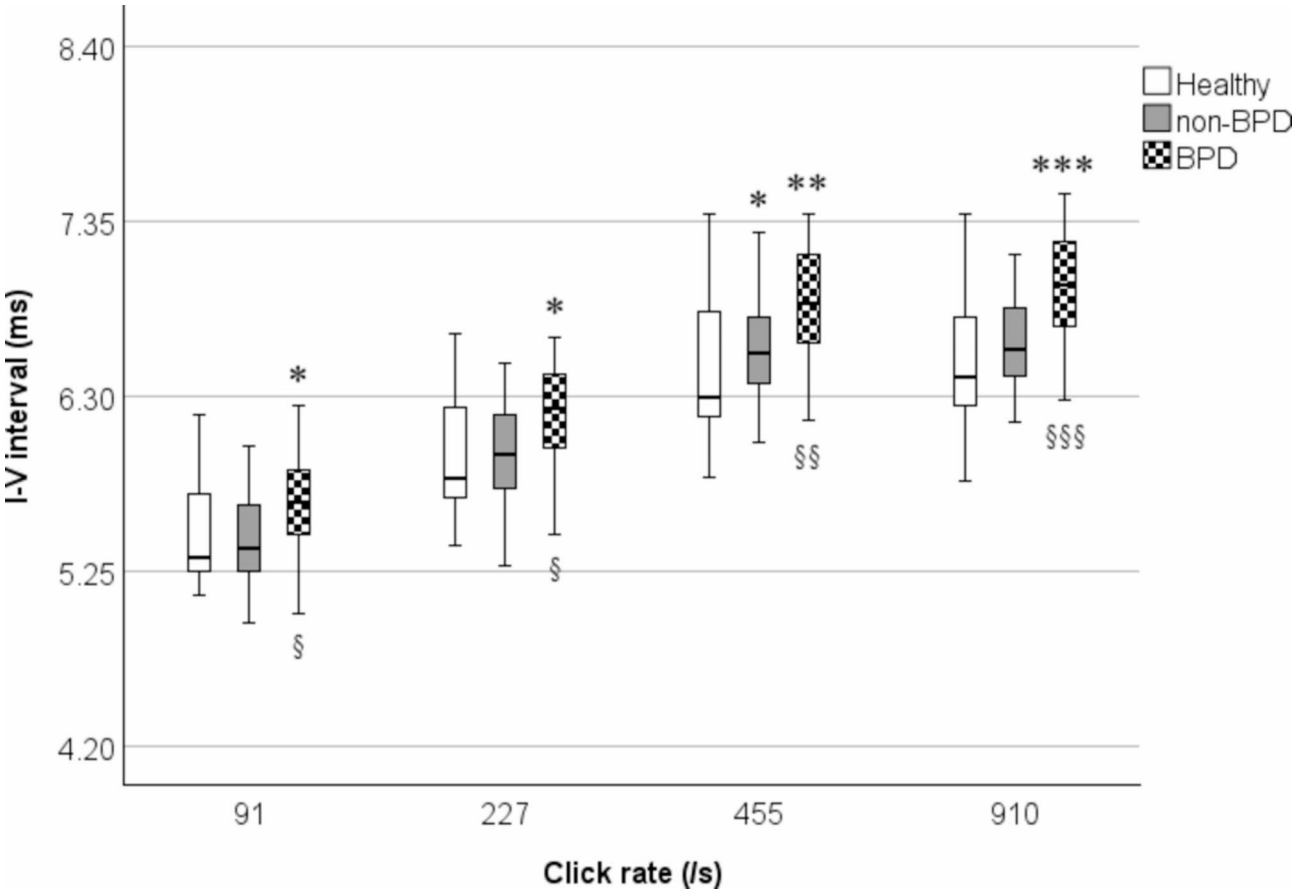

Fig. 4. Bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER I-V interval, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The interval in BPD group is significantly longer than in healthy and non-BPD groups at all click rates, and the differences increase with increasing click rate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ are the significances for the comparison of BPD and non-BPD groups with healthy group; $^{§}p < 0.05$, $^{§§}p < 0.01$, $^{§§§}p < 0.001$ are the significances for the comparison between BPD and non-BPD groups.

specifically reflecting neural conduction at the central brainstem auditory pathway[10,13,14,17–19]. The significant prolongation of the I-V interval at all click rates in babies with BPD indicates major neural conduction impairment of the auditory brainstem at the time when BPD is diagnosed. Previous studies showed that babies with BPD are at an increased risk of impairment in cerebral cortical grey matter growth and white matter damage[20–22]. The present study shows that at the time of diagnosing BPD at PCA 36 weeks VLBW babies with BPD already have clear neural conduction impairment in the auditory brainstem. The MLS BAER abnormalities tended to be more pronounced as the grading of BPD increases. However, because of the relatively small number of babies in each grade, we were unable to performe sound statistical analysis.

The I-III and III-V intervals, the two smaller components of the I-V interval, reflect functional status, specifically neural conduction, at the more peripheral or caudal and more central or rostral regions of the auditory brainstem, respectively[13,14,23]. The prolongation of the two smaller intervals in the BPD babies indicates that both caudal and rostral regions of the auditory brainstem are impaired in babies with BPD at the time when BPD is diagnosed at PCA 36 weeks, i.e., shortly before term age. The prolongation of the III-V interval was more significant than in the I-III interval, suggesting that the neural conduction impairment at rostral or more central regions of the auditory brainstem is more significant than at caudal or more peripheral brainstem regions.

During the maturation, particularly in the preterm period, BAER latencies and interpeak intervals primarily reflect nerve conduction velocity associated with axonal diameter, myelination and synaptic function along the brainstem auditory pathway[14,17–19,24,25]. Other factors, such as neural orientation and synchronization, are also involved. The components and measurements of MLS BAER change with myelination and synaptic maturation[13,14]. The prolongation of wave V latency and particularly interpeak intervals in our BPD babies reflects poor myelination and synaptic dysfunction in the auditory brainstem during the development of BPD, resulting in delayed neural conduction[10,14,26].

The MLS BAER abnormalities in our BPD babies were generally increased with the increase in click rate. The significant increase in the slope for I-V interval-rate function in the BPD babies suggests an increased click rate-dependent change in the auditory brainstem. The click rate-dependent change primarily reflects neural synchronization and the metabolic status of auditory neurons and, in particular, neural processes concerning

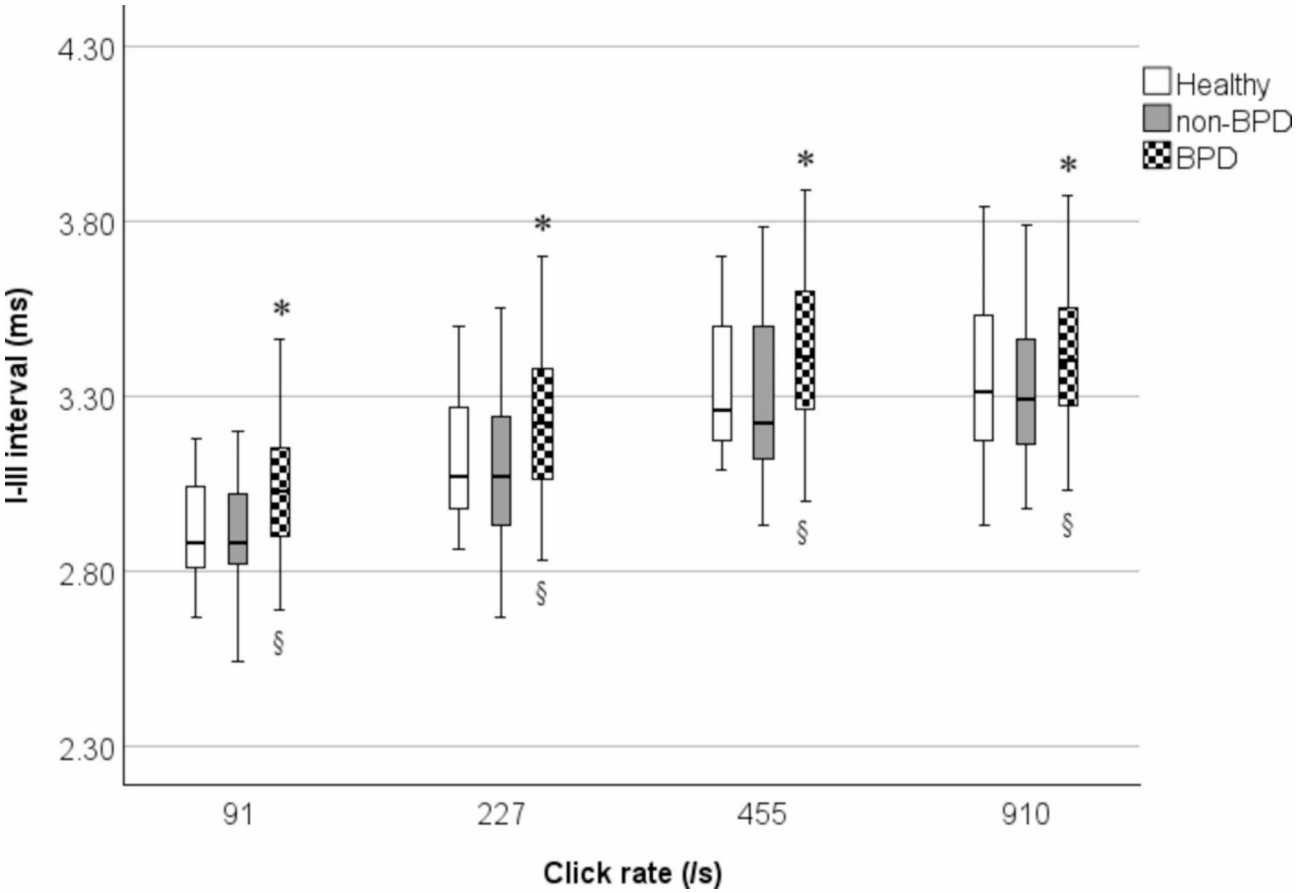

Fig. 5. Boxplot (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER I-III interval, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The interval in BPD group is significantly longer than in healthy and non-BPD groups at all click rates. *$p < 0.05$ is the significance for the comparison of BPD with healthy groups.

the efficacy of central auditory synaptic transmission in the auditory brainstem following the presentation of a physiological/temporal challenge[13,14]. As an important index of synaptic function, the efficacy of synaptic transmission is related to the mechanisms for synthesis, release and uptake of neurotransmitters that play a crucial role in the regulation of neural development as distinct from their differentiated function as neural signal modulators[13,16,27–29]. It appears that neonatal BPD disturbs the metabolism of neurons and depresses the electrophysiological function of synapses in transmitting developmental as well as regulatory signals between neurons in the immature auditory brainstem. The increased click rate-dependent change in our BPD babies implies decreased or impaired efficacy of brainstem synaptic transmission in BPD, or a decreased ability of central auditory neurons to recover in time to transmit the next stimulus-evoked response. Central auditory neurons in BPD are less tolerable to the increase in physiological/temporal challenge of acoustic stimulation. The significant increase in the slope for III-V interval-rate function, with a relatively normal slope for I-III interval-rate function, implies that the decreased or impaired synaptic efficacy occurs mainly at the rostral regions of the brainstem in BPD babies at the time of BPD being diagnosed at PCA 36 weeks.

The pathophysiology of brainstem neural conduction impairment in BPD is multifactorial

It has been well recognized that brain damage in neonatal BPD is complex and mostly due to multiple risk factors[1,2,20,30]. During the development of BPD, the babies often experience intermittent hypoxic episodes. The resultant chronic and sublethal hypoxia plays a major role in brain damage and neurological impairments in neonatal BPD. In experimental animals, chronic hypoxia led to structural, neurochemical and functional alterations or impairment in the immature brain[26,31–33]. Chronic and sublethal hypoxia can also result in severe impairments in corticogenesis, as evidenced by reduced cerebral volume in many regions of the brain in neonatal BPD babies, and a significant decrease in subcortical white matter in the developing brain[22,31]. Glia could be significantly reduced[33].

Brainstem auditory neurons are sensitive to severe hypoxemia[26,34]. During the development of neonatal BPD, frequent episodes of hypoxemia or prolonged hypoxemia can affect the functional status and maturation of auditory neurons in the brainstem[10,26]. In the present study, the BPD babies manifested major abnormalities in MLS BAER components and variables that reflect neural conduction of the auditory brainstem, suggesting

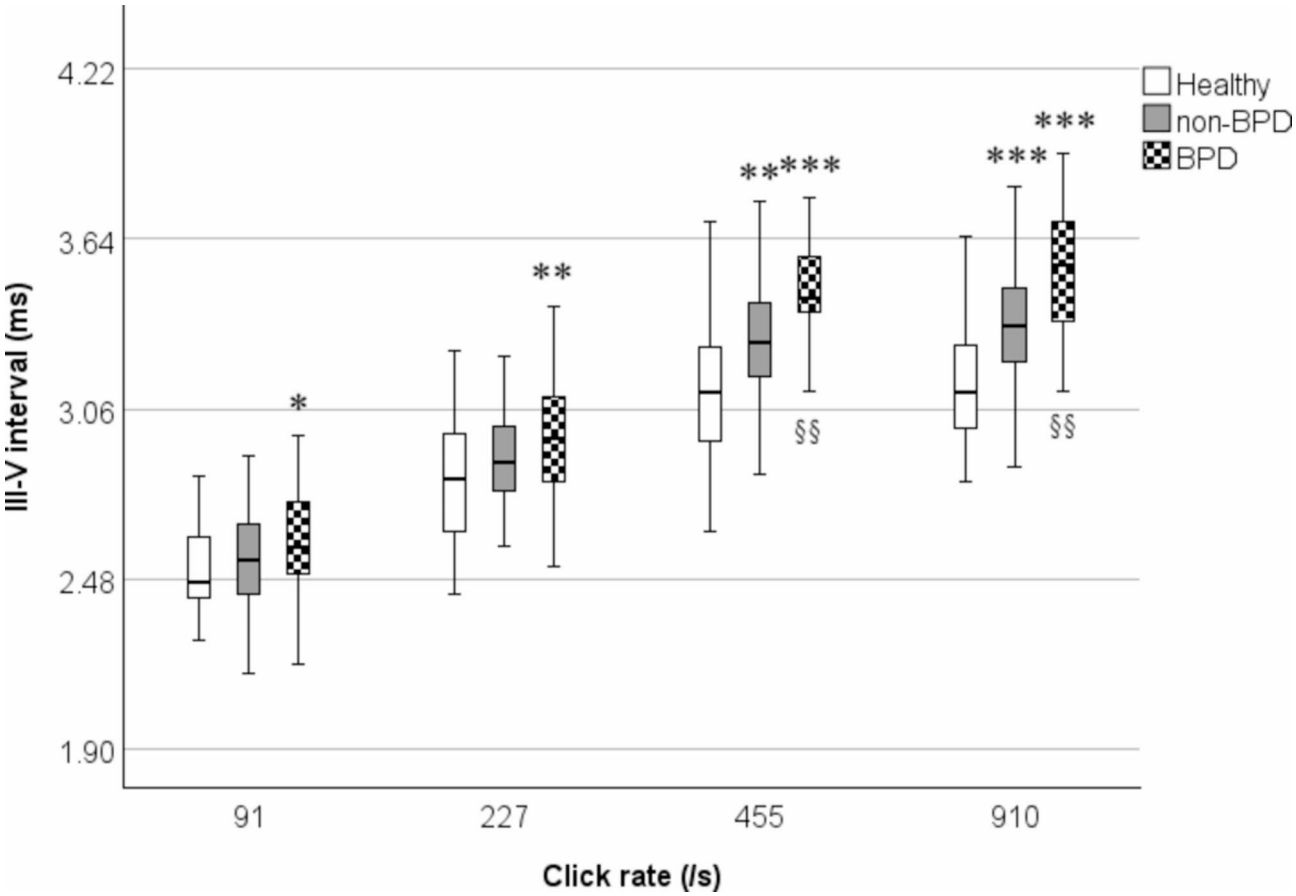

Fig. 6. Boxplot (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER III-V interval, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The interval in BPD group is significantly longer than in healthy and non-BPD groups at all click rates, and the differences increase with increasing click rate. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ are the significances for the comparison of BPD and non-BPD groups with healthy group; $^{§§}p < 0.01$ is the significance for the comparison between BPD and non-BPD groups.

impaired brainstem neural conduction. In our previous experiments in rats raised in chronic sublethal hypoxia, there was a marked reduction in the staining for myelin basic protein and patchy distribution in the residual myelination at the central and upper regions of the brainstem[26]. This histopathological finding was consistent with the significant prolongation of the I–V and particularly III–V intervals in these animals. This is also comparable to the MLS BAER findings in our BPD babies in the present study.

The multifactorial nature of the disease process makes BPD a particularly challenging neonatal condition to prevent and treat[35–38]. In addition to chronic hypoxemia, some other factors associated with neonatal BPD may also contribute to the impairment in brainstem neural conduction, such as respiratory distress syndrome, patent ductus arteriosus, disrupted alveolar and capillary development, pulmonary interstitial emphysema, oxygen toxicity, perinatal infection and inflammation, and genetic susceptibility[1,2,30,36]. These unfavorable conditions can directly or indirectly adversely affect the immature auditory brainstem, and brain development in general, as suggested by the smaller head circumference in our BPD babies.

Differences from the brainstem auditory impairment at term age

The first MLS BAER study in BPD babies was carried out at term age with a mean PCA of 40 weeks[10]. The authors found major MLS BAER abnormalities in BPD babies, including significant prolongation of wave V latency and I-V and III-V intervals. The fundamental abnormality was the significant prolongation of the III-V interval, leading to the significant prolongation of wave V latency and I-V intervals. No obvious abnormality was seen in the I-III interval, suggesting relatively intact neural conduction at more peripheral or caudal regions of the auditory brainstem. Therefore, the major neural conduction impairment at term in BPD babies fundamentally occurs at more central or rostral regions of the brainstem.

In the present study at PCA 36 weeks, the BPD babies manifested similar prolongation of wave V latency and I-V and III-V intervals. Moreover, these babies also manifested a significant prolongation of the I-III interval at almost all click rates. Neural conduction in the auditory brainstem of BPD babies at PCA 36 weeks is impaired at both caudal rostral brainstem regions. This is different from the finding at term that the major neural conduction impairment fundamentally occurs in the rostral brainstem regions, with relatively intact conduction in the

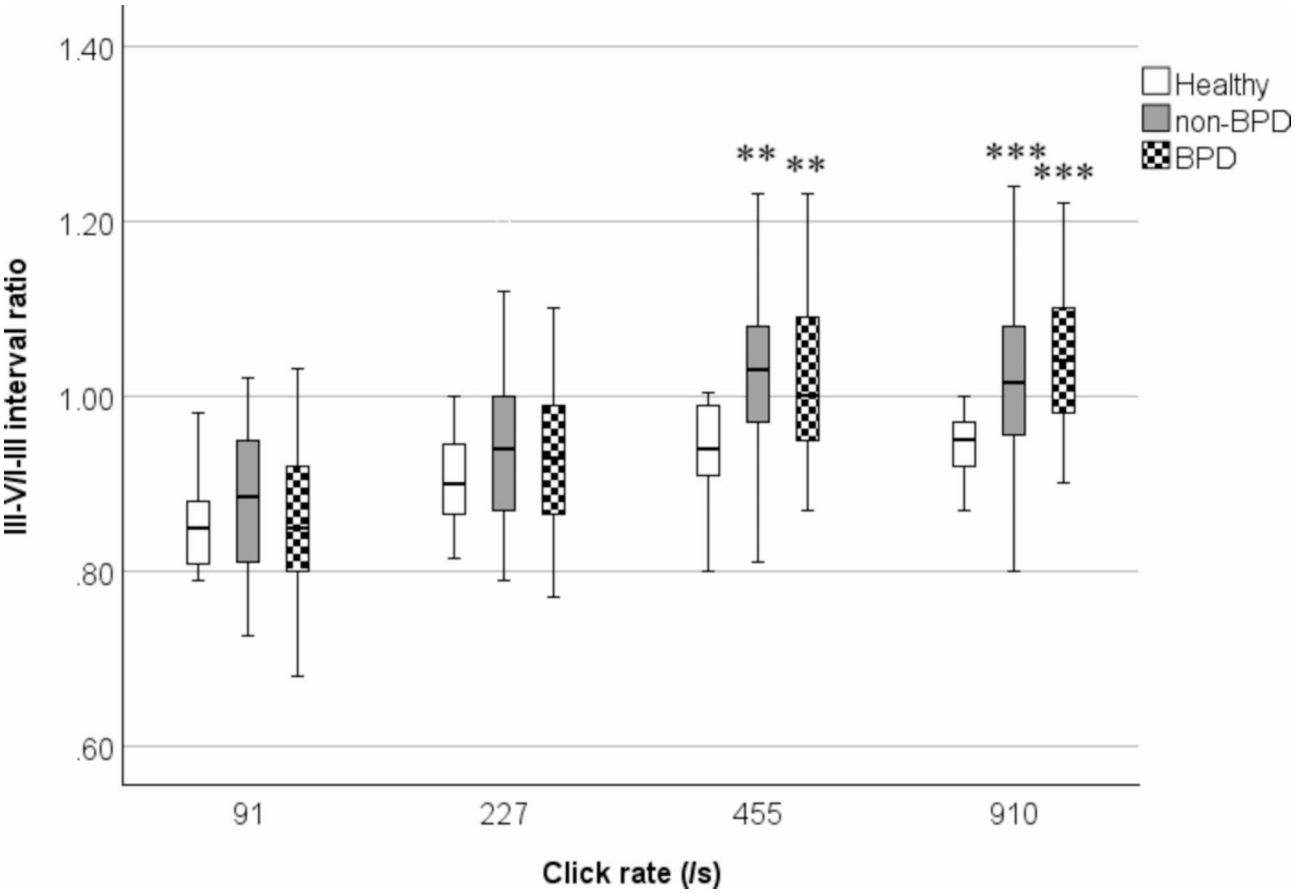

Fig. 7. Boxplot (bold line across the box, median; box, 25th and 75th centile; extensions, the largest and smallest values) of MLS BAER III-V/I-III interval ratio, recorded at 36 weeks of postconceptional age in healthy control, non-BPD and BPD groups in sequence from left to right. The interval ratio in BPD and non-BPD groups is significantly greater than in healthy group at higher click rates. $^{**}p < 0.01$, $^{***}p < 0.001$ are the significances for the comparison of BPD and non-BPD groups with healthy group.

MLS BAER Variable	Subjects	Intercept		Slope (/ decade)[#]		T	*P*
		Mean	SE				P of t-test[§]
I (ms)	Healthy	2.513	0.040	0.006	0.001	4.854	< 0.001
	Non-BPD	2.456	0.042	0.007	0.001	4.863	< 0.001
	BPD	2.452	0.045	0.005	0.002	3.521	< 0.001
III (ms)	Healthy	5.369	0.058	0.017	0.002	8.794	< 0.001
	Non-BPD	5.309	0.054	0.016	0.002	8.831	< 0.001
	BPD	5.428	0.049	0.015	0.002	9.016	< 0.001
V (ms)	Healthy	7.765	0.093	0.034	0.003	10.780	< 0.001
	Non-BPD	7.688	0.065	0.037	0.002	17.299	< 0.001
	BPD	7.832	0.064	0.039	0.002	18.051	< 0.001

Table 2. Linear regression between MLS BAER wave latencies and click rate in BPD, non-BPD and healthy control groups. [#]'Decade' represents 10 clicks per second; [§]'P' refers to the evaluation as to whether the slope differs from 0; P values (< 0.001) indicate that the slopes differ significantly from 0, meaning that there is a significant correlation between MLS BAER variable and the rate of clicks.

MLS BAER variable	Subjects	Intercept		Slope (/ decade)[#]		T	*P*
		Mean	SE				P of t-test[§]
I-V (ms)	Healthy	5.254	0.079	0.027	0.003	10.348	< 0.001
	Non-BPD	5.231	0.058	0.030	0.002	15.690	< 0.001
	BPD	5.375	0.058	0.034	0.002	17.138	< 0.001
I-III (ms)	Healthy	2.852	0.040	0.011	0.001	8.070	< 0.001
	Non-BPD	2.846	0.036	0.009	0.001	7.730	< 0.001
	BPD	2.972	0.040	0.010	0.001	7.065	< 0.001
III-V (ms)	Healthy	2.398	0.046	0.017	0.002	10.715	< 0.001
	Non-BPD	2.387	0.042	0.021	0.001	15.136	< 0.001
	BPD	2.402	0.043	0.024	0.001	16.873	< 0.001
III-V/I-III ratio	Healthy	0.844	0.011	0.002	0.004	5.618	< 0.001
	Non-BPD	0.846	0.017	0.004	0.006	7.375	< 0.001
	BPD	0.822	0.018	0.005	0.006	7.732	< 0.001

Table 3. Linear regression between MLS BAER interpeak intervals and click rate in BPD, non-BPD and healthy control groups. [#]'decade' represents 10 clicks per second; [§]'P' refers to the evaluation as to whether the slope differs from 0; P values (<0.001) indicate that the slopes differ significantly from 0, meaning that there is a significant correlation between MLS BAER variable and the rate of clicks.

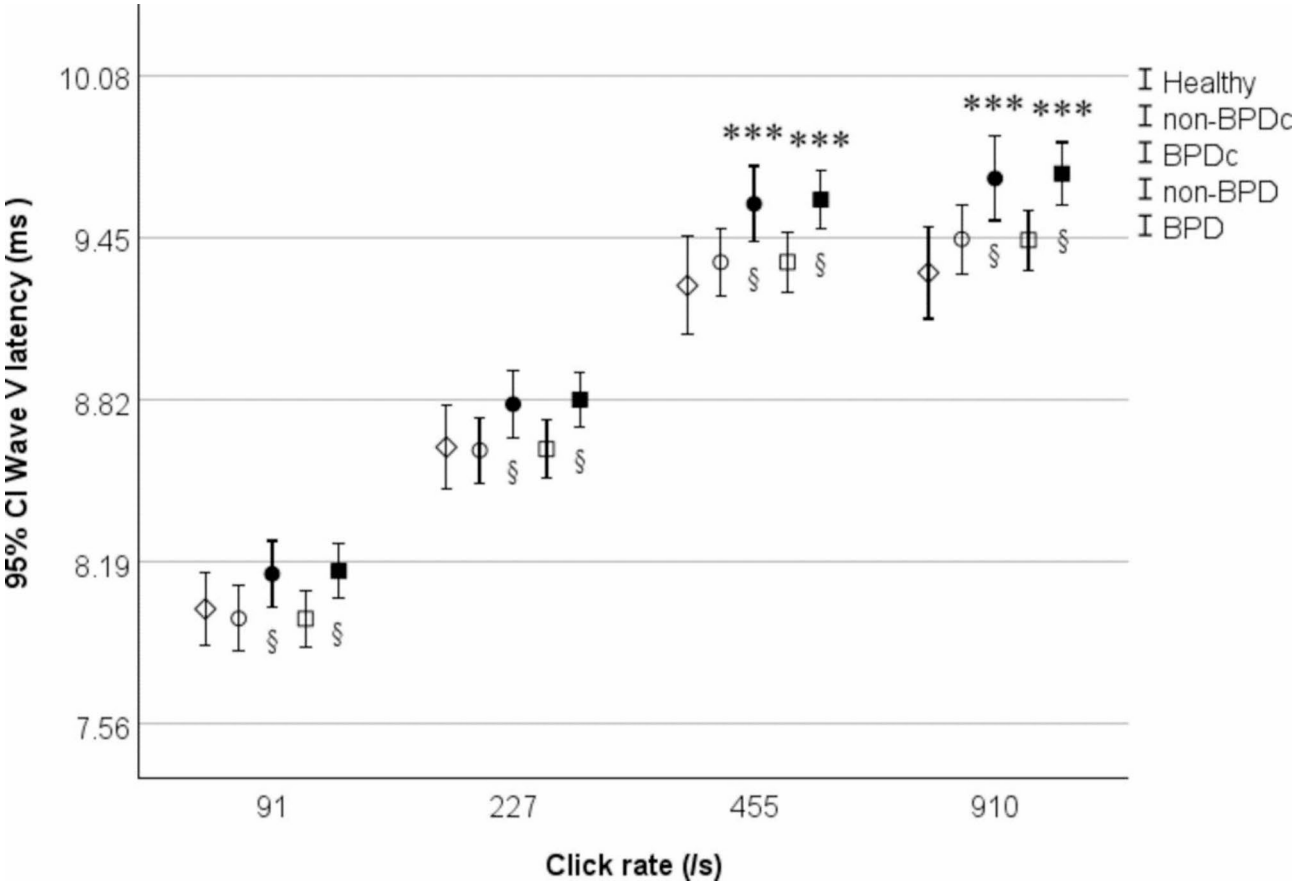

Fig. 8. Means and standard errors of MLS BAER wave V latency, recorded at 36 weeks of postconceptional age in healthy control, non-BPDc (without IVH and PVL), BPDc (without IVH and PVL), non-BPD and BPD groups in sequence from left to right. The latency in both BPDc and BPD groups is significantly longer than in healthy group at 455 and 910/s clicks. $^{***}p < 0.001$ is the significance for the comparison of BPD and BPDc groups with healthy group; $^{\S}p < 0.05$ is the significance for the comparison between BPDc and non-BPDc groups and between BPD and non-BPD groups. No significant differences exist in wave V latency between BPDc and BPD groups and between non-BPDc and non-BPD groups at any click rates.

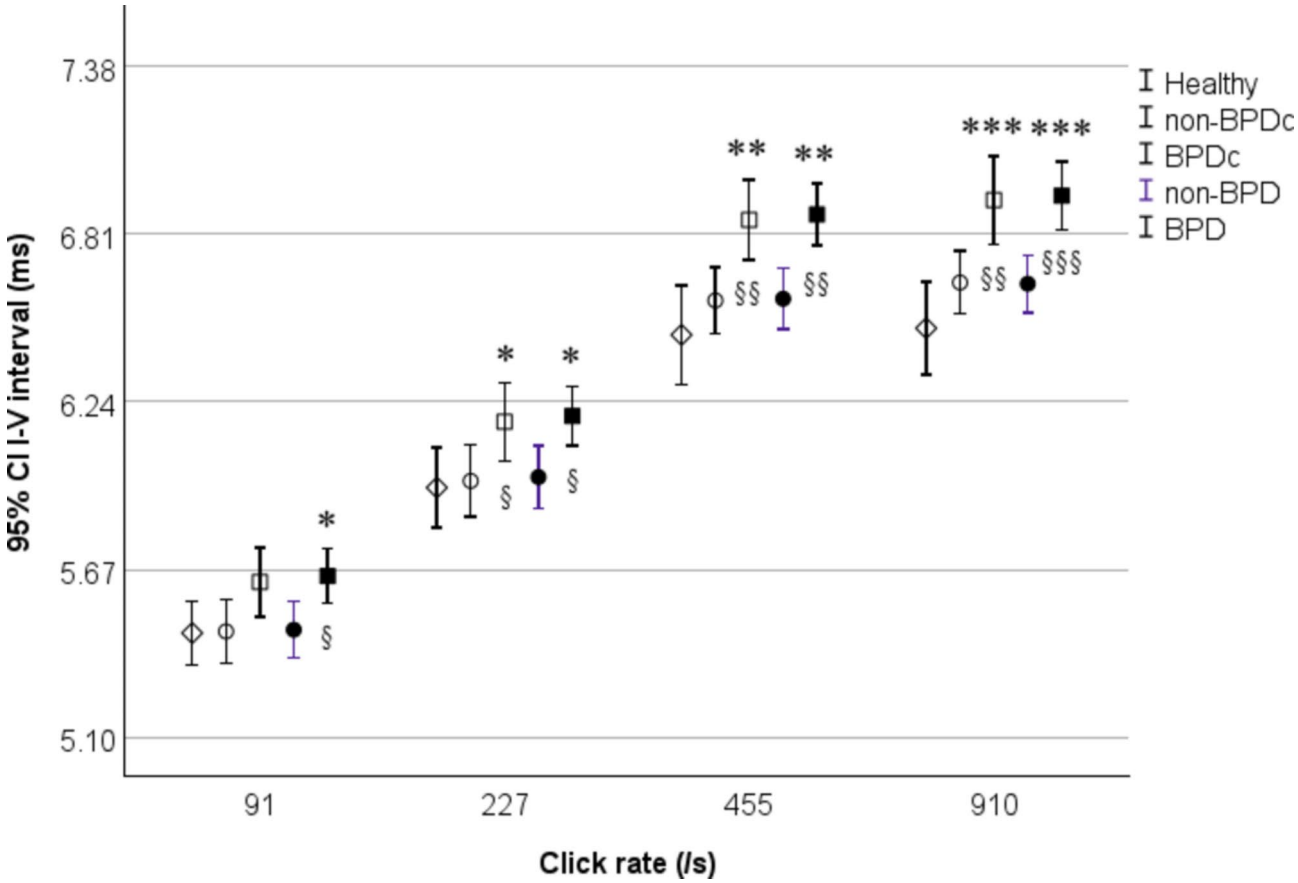

Fig. 9. Means and standard errors of MLS BAER I-V interval, recorded at 36 weeks of postconceptional age in healthy control, non-BPDc (without IVH and PVL), BPDc (without IVH and PVL), non-BPD and BPD groups in sequence from left to right. The interval in both BPDc and BPD groups is significantly longer than in healthy group at higher click rates. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ are the significances for the comparison of BPD and BPDc groups with healthy group; $^{\S}p<0.05$, $^{\S\S}p<0.01$, $^{\S\S\S}p<0.001$ are the significances for the comparison between BPDc and non-BPDc groups and between BPD and non-BPD groups. No significant differences exist in I-V interval between BPDc and BPD groups and between non-BPDc and non-BPD groups at any click rates.

caudal brainstem regions[10]. It is apparent that the major neural conduction impairment in the rostral brainstem regions in BPD babies persists both before term and at term, whereas the impairment in the caudal brainstem regions is present before term and alleviated at term date.

Major differences in brainstem neural conduction between VLBW babies diagnosed with BPD and those with no BPD at the time of diagnosis

The comparison among the BPD, non-BPD and healthy control groups showed significant differences in MLS BAER at the time when diagnosing BPD at PCA 36 weeks. A further detailed comparison between any two groups of babies revealed that the differences were predominantly produced by the major abnormalities in the BPD group. Compared with healthy controls, VLBW babies who were diagnosed with BPD manifested major MLS BAER abnormalities, including significant prolongation of wave V latency and all I-V, I-III, and III-V intervals at all click rates. However, those who were not diagnosed with BPD, i.e., non-BPD babies, manifested only minor abnormalities, including moderate prolongation of the I-V and III-V intervals at higher click rates, suggesting the limited adverse effect of very preterm birth and VLBW on brainstem neural conduction.

The direct comparison of MLS BAER between the BPD and non-BPD babies revealed major differences. Wave V latency and all I-V, I-III and III-V intervals in the BPD babies were significantly longer than those in the non-BPD babies. Clearly, there is major neural conduction impairment in the brainstem if VLBW babies are complicated with BPD, but only minor impairment if VLBW babies are not complicated with BPD. This further supports and confirms that neonatal BPD constitutes a major risk factor for neural conduction impairment of the brainstem in VLBW babies.

In our non-BPD babies, the I-III interval was similar to that in the healthy controls at all click rates. In the BPD babies, however, this interval was significantly longer than in the healthy controls, and also longer than in the non-BPD babies at almost all click rates used. These results suggest that the caudal brainstem regions are essentially normal in the babies without BPD, but are impaired in the babies with BPD. The significantly

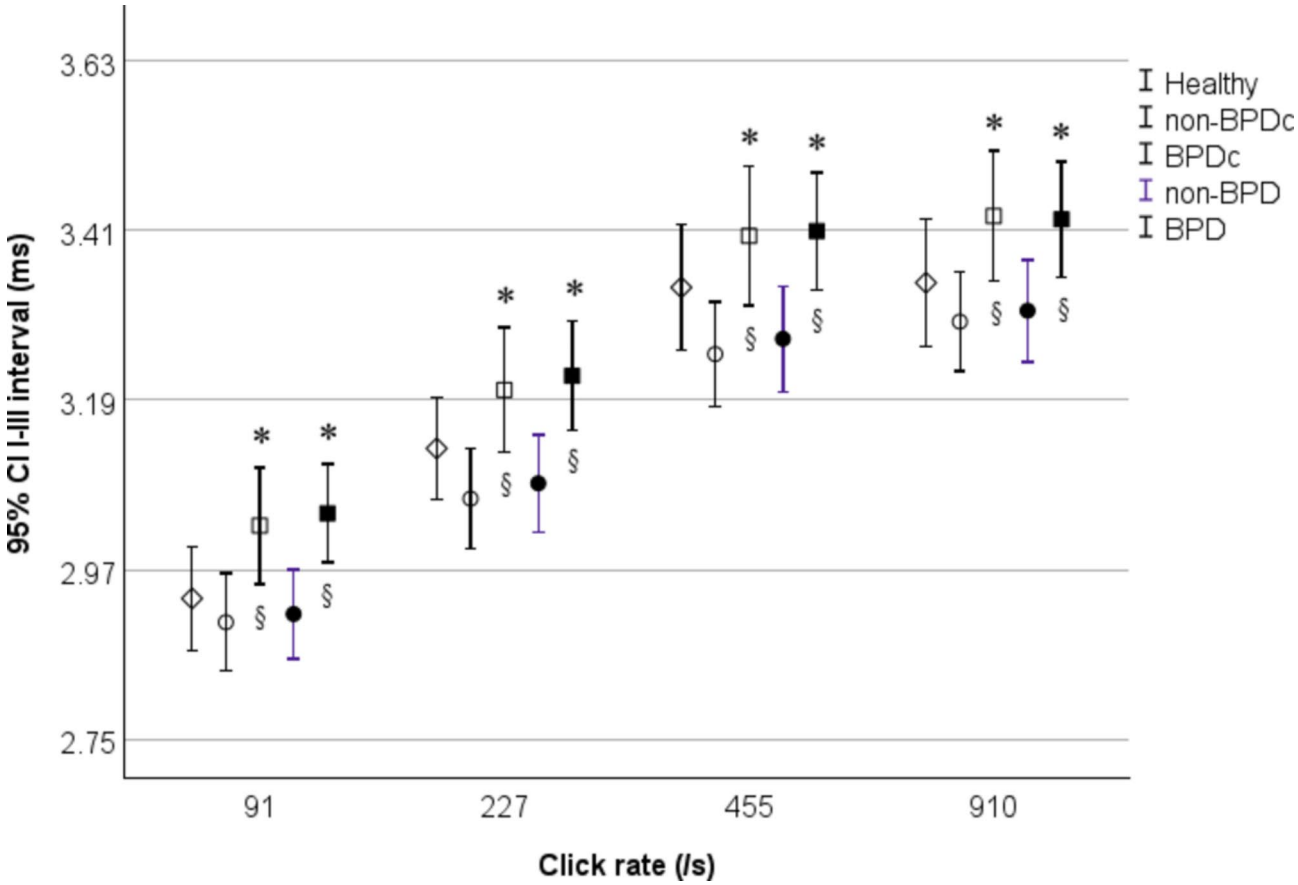

Fig. 10. Means and standard errors of MLS BAER I-III interval, recorded at 36 weeks of postconceptional age in healthy control, non-BPDc (without IVH and PVL), BPDc (without IVH and PVL), non-BPD and BPD groups in sequence from left to right. The interval in both BPDc and BPD groups is significantly longer than in healthy group at all click rates. $^{*}p < 0.05$ is the significance for the comparison of BPD and BPDc groups with healthy group; $^{\S}p < 0.05$ is the significance for the comparison between BPDc and non-BPDc groups and between BPD and non-BPD groups. No significant differences exist in I-III interval between BPDc and BPD groups and between non-BPDc and non-BPD groups at any click rates.

prolonged III-V in the BPD group than in the non-BPD group implies that neural conduction impairment at the rostral brainstem regions is much more severe in babies with BPD than in those without BPD.

The click rate-dependent change in brainstem auditory neurons was significantly increased in our BPD babies, but was only slightly or moderately increased in the non-BPD babies. Both the slopes for I-V and III-V interval-rate functions in the BPD babies were moderately greater than in the non-BPD babies. Therefore, the decrease in the efficacy of brainstem synaptic transmission in BPD or the decrease in the ability of central auditory neurons to recover in time to transmit the next stimulus-evoked response is more significant in BPD babies than in non-BPD babies.

Additionally, we re-analysed the MLS BAER data after excluding the small numbers of babies with IVH and PVL in both the BPD and non-BPD groups to assess any confounding effects of IVH and PVL on the MLS BAER data. The results showed that the MLS BAER data obtained, particularly wave V latency and the I-V, I-III and III-V intervals, were similar to those when all babies were included, without any pronounced differences. None of the MLS BAER variables differed significantly between when the babies with IVH and PVL were included and when these babies were excluded. Clearly, the small numbers of babies with IVH and PVL in the BPD and non-BPD groups did not impose any significant confounding effects on the MLS BAER results in either the BPD or non-BPD groups.

Conclusions

At PCA 36 weeks at which the diagnosis of neonatal BPD was made in VLBW babies, major MLS BAER abnormalities were found in the babies who were diagnosed with BPD, whereas only minor abnormalities were seen in the babies who were not diagnosed with BPD. Both caudal and, in particular, rostral regions of the auditory brainstem are impaired in VLBW babies with BPD, but only rostral brainstem regions are mildly impaired in those without BPD. The marked differences in MLS BAER between BPD and non-BPD help differentiate the major brainstem impairment in BPD babies from the minor impairment in non-BPD babies. The development of BPD at and before PCA 36 weeks has a detrimental effect on the early maturation of the auditory brainstem,

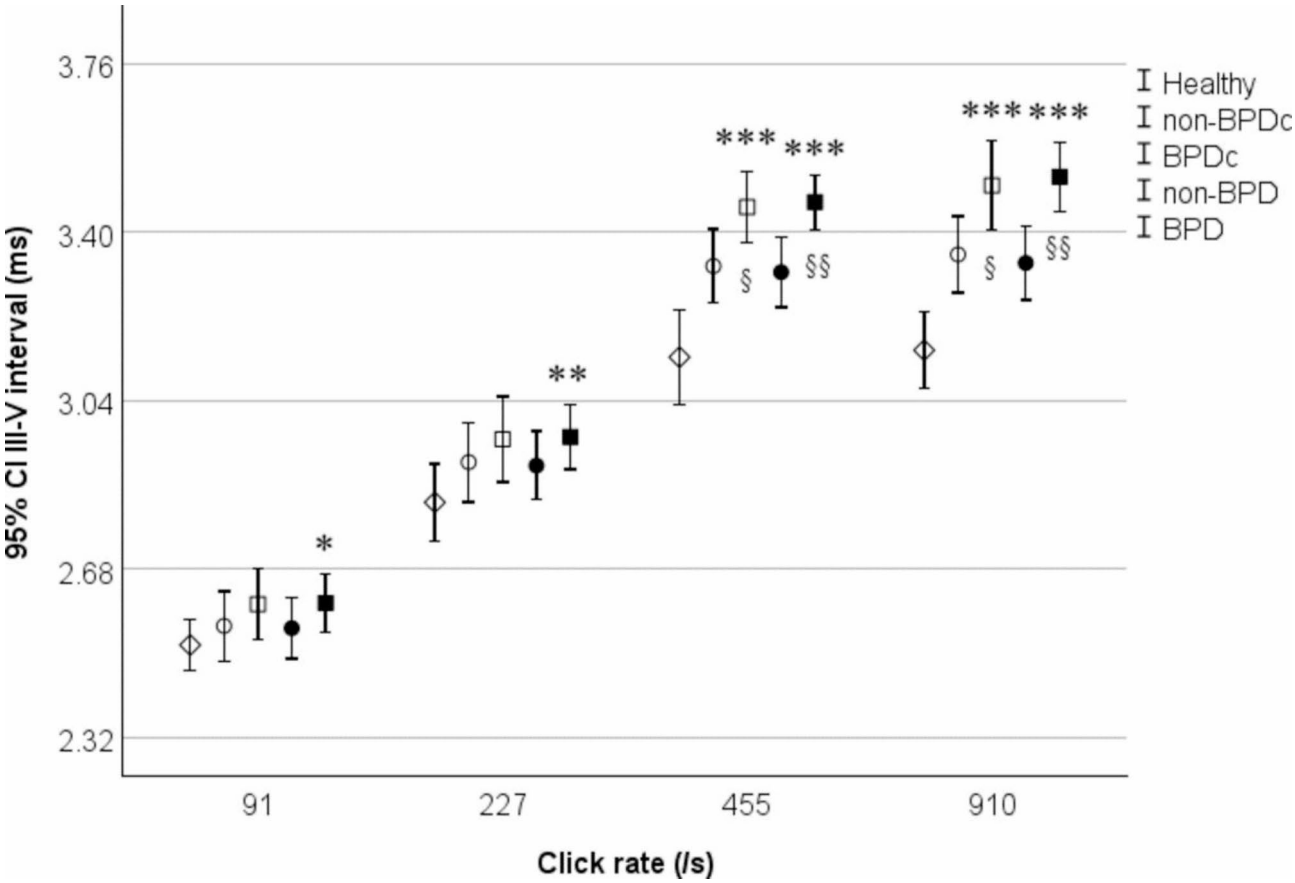

Fig. 11. Means and standard errors of MLS BAER III-V interval, recorded at 36 weeks of postconceptional age in healthy control, non-BPDc (without IVH and PVL), BPDc (without IVH and PVL), non-BPD and BPD groups in sequence from left to right. The interval in both BPDc and BPD groups is significantly longer than in healthy group at higher click rates. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ are the significances for the comparison of BPD and BPDc groups with healthy group; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ are the significances for the comparison between BPDc and non-BPDc groups and between BPD and non-BPDgroups. No significant differences exist in III-V interval between BPDc and BPD groups and between non-BPDc and non-BPD groups at any click rates.

and more widely, the immature brain of VLBW babies. These findings offer new insight into brain damage and neurological impairment in BPD before term age. The major MLS BAER abnormalities in BPD babies might be an additive "biomarker" of brain damage and neurological impairment for VLBW babies with BPD at the time of being diagnosed.

One limitation of this study was that we were unable to follow later neurodevelopment in most of the babies studied. Further research into the relationship of earlier MS BAER findings with later neurodevelopmental outcomes in BPD babies would allow us to assess the possible predictive value of earlier MLS BAER findings for later neurodevelopmental outcomes. This could be achieved by using such as Griffiths Mental Development Scales, BSID Bayley Scales of Infant Development, and the Standardized Infant NeuroDevelopmental Assessment (SINDA), as well as standardized auditory or hearing assessments[39]. It would also be of great interest to explore if there are any MLS BAER abnormalities in BPD babies before PCA 36 weeks. This would allow to understand if brainstem abnormality already occurs even before the diagnosis of BPD is made at PCA 36 weeks.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 7 March 2024; Accepted: 4 February 2025
Published online: 15 May 2025

References

1. Chess, P. R., D'Angio, C. T., Pryhuber, G. S. & Maniscalco, W. M. Pathogenesis of bronchopulmonary dysplasia. *Semin. Perinatol.* **30**, 171–178 (2006).
2. D'Angio, C. T. & Maniscalco, W. M. Bronchopulmonary dysplasia in preterm infants: pathophysiology and management strategies. *Paediatr. Drugs*. **6**, 303–330 (2004).

3. Zysman-Colman, Z., Tremblay, G. M., Bandeali, S. & Landry, J. S. Bronchopulmonary dysplasia – trends over three decades. *Paediatr. Child. Health*. **18**, 86–90 (2013).
4. Doyle, L. W. & Anderson, P. J. Long-term outcomes of bronchopulmonary dysplasia. *Semin Fetal Neonatal Med.* **14**, 391–395 (2009).
5. Karagianni, P. et al. Neuromotor outcomes in infants with bronchopulmonary dysplasia. *Pediatr. Neurol.* **44**, 40–46 (2011).
6. Karemaker, R. et al. Differences in behavioral outcome and motor development at school age after neonatal treatment for chronic lung disease with dexamethasone versus hydrocortisone. *Pediatr. Res.* **60**, 745–750 (2006).
7. Martin, M. et al. Bronchopulmonary dysplasia and neurobehavioural outcomes at birth and 2 years in infants born before 30 weeks. *Arch. Dis. Child. Fetal Neonatal Ed.* **108**, 142–148 (2023).
8. Neubauer, V., Junker, D., Griesmaier, E., Schocke, M. & Kiechl-Kohlendorfer, U. Bronchopulmonary dysplasia is associated with delayed structural brain maturation in preterm infants. *Neonatology* **107**, 179–184 (2015).
9. Short, E. J. et al. Cognitive and academic consequences of bronchopulmonary dysplasia and very low birth weight: 8-year-old outcomes. *Pediatrics* **112**, e359–e366 (2003).
10. Wilkinson, A. R., Brosi, D. M. & Jiang, Z. D. Functional impairment of the brainstem in infants with bronchopulmonary dysplasia. *Pediatrics* **120**, 362–371 (2007).
11. Bancalari, E., Claure, N. & Sosenko, I. R. S. Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. *Semin Neonatl.* **8**, 63–71 (2003).
12. Lapcharoensap, W. et al. Hospital variation and risk factors for bronchopulmonary dysplasia in a population-based cohort. *JAMA Pediatr.* **169**, e143676 (2015).
13. Jiang, Z. D. Maximum length sequence technique improves detection of neuropathology involving infant brainstem. In: (eds Lawson, P. N. & McCarthy, E. A.) Pediatric Neurology. New York: Nova Science; 1–38. (2012).
14. Jiang, Z. D. Evoked potentials in pediatric brainstem lesions. In: (eds Galloway, G.) Clinical Neurophysiology in Pediatrics: A Practical Approach to Neurodiagnostic Testing and Management. New York: Demos Medical Publishing, LLC; 187–213. (2015).
15. Wilkinson, A. R. & Jiang, Z. D. Brainstem auditory evoked response in neonatal neurology. *Semin Fet Neonatol Med.* **11**, 444–451 (2006).
16. Jiang, Z. D., Wang, C., Ping, L. L. & Yin, R. Altered maturation in brainstem neural conduction in very premature babies with fetal growth restriction. *Pediatr. Res.* **94**, 1472–1479 (2023).
17. Eggermont, J. J. Auditory brainstem response. *Handb. Clin. Neurol.* **160**, 451–464. https://doi.org/10.1016/B978-0-444-64032-1.00030-8 (2019).
18. Møller, A. R. Neural generators for auditory brainstem evoked potentials. In: (eds Burkard, R. F., Don, M. & Eggermont, J. J.) Auditory Evoked Potentials: Basic Principles and Clinical Application. Baltimore: Lippincott Williams and Wilkins. 336–354. (2007).
19. Ponton, C. W., Moore, J. K. & Eggermont, J. J. Auditory brain stem response generation by parallel pathways: differential maturation of axonal conduction time and synaptic transmission. *Ear Hear.* **17**, 402–410 (1996).
20. Gagliardi, L., Bellù, R., Zanini, R. & Dammann, O. Bronchopulmonary dysplasia and brain white matter damage in the preterm infant: a complex relationship. *Paediatr. Perinat. Epidemiol.* **23**, 582–590 (2009).
21. Murphy, B. P. et al. Impaired cerebral cortical gray matter growth after treatment with dexamethasone for neonatal chronic lung disease. *Pediatrics* **107**, 217–221 (2001).
22. Thompson, D. K. et al. Perinatal risk factors altering regional brain structure in the preterm infant. *Brain* **130**, 667–677 (2007).
23. Jiang, Z. D., Brosi, D., Wu, Y. Y. & Wilkinson, A. R. Relative maturation of the peripheral and central regions of the auditory brainstem from preterm to term and the influence of preterm birth. *Pediatr. Res.* **65**, 657–662 (2009).
24. Moore, J. K. & Linthicum, F. H. Jr The human auditory system: a timeline of development. *Int. J. Audiol.* **46**, 460–478 (2007).
25. Moore, J. K., Perazzo, L. M. & Braun, A. Time course of axonal myelination in the human brainstem auditory pathway. *Hear. Res.* **87**, 21–31 (1995).
26. Jiang, Z. D. Damage of chronic sublethal hypoxia to the immature auditory brainstem. In: (eds Fiedler, D. & Krause, R.) Deafness, Hearing Loss, and the Auditory System. New York, USA: Nova Science; 159–180. (2010).
27. Jiang, Z. D. & Ping, L. L. Functional integrity of rostral regions of the immature brainstem is impaired in extremely preterm babies. *Clin. Neurophysiol.* **127**, 1581–1588 (2016).
28. Jiang, Z. D., Pin, L. L. & Wilkinson, A. R. Functional abnormality of the auditory brainstem in high-risk late preterm infants. *Clin. Neurophysiol.* **123**, 993–1001 (2012).
29. Lasky, R. E. Rate and adaptation effects on the auditory evoked brainstem response in human newborns and adults. *Hear. Res.* **111**, 165–176 (1997).
30. Gien, J. & Kinsella, J. P. Pathogenesis and treatment of bronchopulmonary dysplasia. *Curr. Opin. Pediatr.* **23**, 305–313 (2011).
31. Ment, L. R., Schwartz, M., Makuch, R. W. & Stewart, W. B. Association of chronic sublethal hypoxia with ventriculomegaly in the developing rat brain. *Dev. Brain Res.* **111**, 197–203 (1998).
32. Raman, L. et al. In vivo effect of chronic hypoxia on the neurochemical profile of the developing rat hippocampus. *Brain Res. Dev. Brain Res.* **156**, 202–209 (2005).
33. Schwartz, M. L. S. et al. Chronic neonatal hypoxia leads to long term decreases in the volume and cell number of the rat cerebral cortex. *Semin Perinatol.* **28**, 379–388 (2004).
34. Inagaki, M. et al. Hypoxia-induced ABR changes and heat shock protein expression in the pontine auditory pathway of young rabbits. *Brain Res.* **757**, 111–118 (1997).
35. Iyengar, A. & Davis, J. M. Drug therapy for the prevention and treatment of bronchopulmonary dysplasia. *Front. Pharmacol.* **6**, 12 (2015).
36. Kugelman, A. & Durand, M. A comprehensive approach to the prevention of bronchopulmonary dysplasia. *Pediatr. Pulmonol.* **46**, 1153–1165 (2011).
37. Pierro, M., Ciarmoli, E. & Thébaud, B. Bronchopulmonary dysplasia and chronic lung disease: Stem Cell Therapy. *Clin. Perinatol.* **42**, 889–910 (2015).
38. Tin, W. & Wiswell, T. E. Adjunctive therapies in chronic lung disease: examining the evidence. *Semin. Fetal Neonatal. Med.* **13**, 44–52 (2008).
39. Bower, C., Reilly, B. K., Richerson, J. & Hecht, J. L. Hearing Assessment in infants, children, and adolescents: recommendations beyond neonatal screening. *Pediatrics* **152**, e2023063288. https://doi.org/10.1542/peds.2023-063288 (2023).

Acknowledgements

We thank the doctors, researchers and nurses at the Neonatal Division of Children's Hospital for their assistance in recruiting subjects and collecting data. The work was partly supported by the Medical Sciences Division, University of Oxford, UK.

Author contributions

Z.D.J. designed and overall supervised the project and was responsible for the final version of the manuscript. R.Y. made a major contribution to recording MLS BAER and collecting clinical data. J.K.J was responsible for

analysing data and writing the manuscript. C.W. contributed to collecting and analysing clinical and MLS BAER data.

Declarations

Competing interests
The authors declare no competing interests.

Informed consent
Informed parental consent was obtained for each baby studied.

Additional information
Correspondence and requests for materials should be addressed to Z.D.J.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.