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Effect of bilirubin on visuocortical development in preterm infants

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OBJECTIVE: To determine if visuocortical development in premature infants with high bilirubin levels is more adversely affected than that in full-term infants.

STUDY DESIGN: 57 preterm infants were managed using institutional guidelines for hyperbilirubinemia. At 12-months corrected age, Vernier acuity, contrast sensitivity, and grating acuity measured using the sweep visual evoked potential (sVEP) were correlated to total serum/plasma bilirubin (TSB) levels in the first week of life.

RESULT: As TSB levels increased, Vernier acuity worsened in infants <34 weeks' gestation compared with those >34 to <37 weeks' gestation ($p < 0.001$). Contrast sensitivity varied as a function of TSB levels (Spearman correlation 0.63, $p < 0.001$). Grating acuity was unaffected.

CONCLUSION: Vernier acuity in preterm infants <34 weeks' gestation is more vulnerable to the effects of bilirubin, suggesting that the extrastriate visual cortex is primarily affected by bilirubin. Therefore, guidelines for management of hyperbilirubinemia in preterm infants (<34 weeks' gestation) should be revised.

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INTRODUCTION

Visual perception, visual information interpretation, environmental navigation, and cognitive and social behaviors rely on visual cues optimized during neurodevelopmental maturation and visual signal processing [1, 2]. Assessments of visual neuromaturation during infancy have been studied using visual evoked potentials (VEP) and reported long-lasting negative effects on visuocortical development among term neonates with hyperbilirubinemia [3, 4]. This is consistent with findings from other parts of the nervous system, e.g., impairment of auditory signal processing caused by bilirubin neurotoxicity [5–7].

Neonatal hyperbilirubinemia is an imbalance between bilirubin production (e.g., often from hemolysis) and elimination (e.g., from conjugation defects) and usually benign. However, when the physiologic postnatal surge in total serum/plasma bilirubin (TSB) levels is exaggerated or the neuroprotective role of albumin-bilirubin binding is overwhelmed, unbound bilirubin can enter the brain, resulting in devastating neurological sequelae [8–10]. Serious adverse outcomes have been minimized by clinical implementation of the 2004 American Academy of Pediatrics (AAP) Guideline for the Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks Gestation [11, 12]. For those < 35 weeks, experience-based guidelines exist, but are partially defined by expert consensus and not proven to be protective [13–15]. Premature neonates are vulnerable due to an immature blood-brain barrier and more susceptible to neurotoxicity at lower TSB levels compared with those born full term [8, 10].

To expand our understanding of bilirubin neurotoxicity, we explored the possibility of preterm infants being even more vulnerable to visuocortical impairment due to bilirubin exposure [3, 4]. Clinical practice recommends aggressive interventions to manage preterm infants with excess TSB levels [12–14, 16, 17]. Yet, the safety of these approaches has not been prospectively evaluated except in two National Institute of Child Health and Development Neonatal Research Network phototherapy trials in extremely low birthweight infants (<1000 grams or < 28 weeks' gestation) [18–20]. Here we assessed visuocortical development or its impairment in preterm infants (<37 weeks' gestation) exposed to bilirubin using sweep VEP (sVEP) determinations.

MATERIALS/SUBJECTS AND METHODS

This was a prospective cohort study at the neonatal intensive care units (NICUs) at two tertiary hospitals in California. The study, conducted from July 20, 2020 to December 6, 2023, was approved by the Institutional Review Boards at Sutter Health and The Smith-Kettlewell Eye Research Institute. Signed parental informed consents were obtained.

Patient enrollment

Newborns born < 37 weeks' gestation at two California Sutter Health nurseries (Alta Bates Summit Medical Center, Berkeley, CA and California Pacific Medical Center, San Francisco, CA) were

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recruited consecutively. Excluded infants were those with known neurological diseases (e.g., intraventricular hemorrhage, meningitis, brain anomalies, or hypoxia/ischemia), thyroid dysfunction, or structural eye abnormalities. Clinical information included gestational age (GA), birthweight, sex, TSB levels, and phototherapy use. Infants were classified as: extremely preterm: GA < 28 weeks' gestation; very to moderate preterm: 28 to < 34 weeks' gestation; and late preterm: 34 to < 37 weeks' gestation. Supplementary Fig. 1 is the consort diagram describing enrollment and retention.

TSB levels and risk assessment

TSB levels were measured using the diazo method at both clinical laboratories. Per institutional practices, thresholds for initiating phototherapy were defined using the web-based Premie BiliRecs™ CDS tool (pbr.stanfordchildrens.org) for infants aged 27 to < 35 weeks' gestation [15]. For infants ≥ 35 to < 37 weeks' gestation, 2004 AAP Guideline [11] and the 2009 clarification [12] thresholds were used. Neonates < 27 weeks' gestation were treated at clinician discretion. For this study, we designated infants who required phototherapy as "treated"; whereas, those that did not as "untreated".

Visual threshold measurements

At 12-months corrected age, parents were asked to return for sVEP examinations to assess Vernier acuity, contrast sensitivity, and grating acuity (Supplementary Fig. 2) by scientists masked to the patient's TSB level, name, and GA at birth [3, 4]. The target age at testing of 12-months corrected age is identical to that in our previous term infant study [3] in order to compare differences between the two studies. All measurements were recorded in one sitting. In brief, the three sensory visual stimuli were presented in random order to obviate fatigue from showing each stimulus sequentially. Approximately ten of the same stimuli (Vernier acuity, contrast sensitivity, grating acuity) were shown to the infant seated on a parent's lap one meter away. The infant's attention was maintained by examiners singing and dangling toys in front of the displayed stimulus. Results were signal averaged for each stimulus.

Vernier acuity (in arc minutes) is the ability to discern the small offsets between two line segments, with increasing values reflecting worsening acuity (Supplementary Fig. 2). Vernier acuity (as measured by sVEP) is relatively immature during the first year of life [21]. Worsening Vernier acuity results in problems with motion and movement detection. Contrast sensitivity (percent of control) is the ability to detect slight changes in luminance across space, with increasing values reflecting worsening sensitivity. A reduction in contrast sensitivity results in a diminished ability to see in dim lighting. It is the first of the three functions to mature, with sensitivity being half that of adult sensitivity by three months of age [22, 23]. Grating acuity (in cycles per degree [cpd]) is the ability to detect fine patterns of spatial resolution (lines) and represents the finest grating that can be resolved, with decreasing values reflecting worsening acuity, affecting the ability to see thin lines, and their orientation (e.g., vertical, horizontal). Grating acuity thresholds reach half of adult values by eight months of age [24].

Statistical analyses

All data are expressed as mean ± SD or percentages and ranges. For all non-parametric comparisons, Spearman rank correlation coefficients and 95% confidence intervals (CIs) and linear regressions were calculated. For categorical comparisons, χ^2 tests were used. For all other comparisons, unpaired Student's t test or one-way ANOVAs were applied. p values ≤ 0.05 were deemed statistically significant. All statistical analyses were performed using Prism v9.0 (GraphPad Software., Boston, MA).

Table 1. Patient demographics of infants who completed sVEP exams at the 12 months of age visit ($n = 7$) stratified by each preterm cohort.

	All	<34 weeks	34 to <37 weeks
n	57	29 (51%)	28 (49%)
GA (weeks)	32.9 ± 2.3 (25.0–36.6)	31.1 ± 1.9 (25.0–33.4)	34.7 ± 0.7 (34.0–36.6)
		*$p < 0.001$	
Birthweight (grams)	1855 ± 487 (700–2745)	1553 ± 432 (700–2363)	2168 ± 315 (1660–2745)
		*$p < 0.001$	
Sex			
Male	28 (49%)	14 (48%)	14 (50%)
Female	29 (51%)	15 (52%)	14 (50%)
TSB (mg/dL)	9.9 ± 2.6 (4.1–14.3)	9.6 ± 2.3 (6.3–13.9)	10.3 ± 2.9 (4.1–14.3)
Postnatal Age (days)	3.8 ± 1.5 (2.0–9.0)	3.8 ± 1.7 (2.0–9.0)	3.8 ± 1.3 (2.0–7.0)
Treatment[∂]			
Yes	39 (68%)	25 (86%)	14 (50%)
No	18 (32%)	4 (14%)	14 (50%)
		†$p = 0.003$	
Age at 12-month Visit (months)	11.6 ± 1.5 (8.8–14.6)	11.2 ± 1.3 (8.8–14.5)	12.0 ± 1.6 (9.4–14.6)
	57	29	28
Vernier Acuity (arcmin)	2.17 ± 1.22 (0.72–5.86)	2.36 ± 1.50 (0.82–5.86)	1.97 ± 0.81 (0.72–3.44)
	57	29	28
Contrast Sensitivity (%)	1.91 ± 0.93 (0.63–4.40)	1.83 ± 0.87 (0.63–4.32)	2.01 ± 1.01 (0.81–4.40)
	56	29	27
Grating Acuity (cpd)	17.23 ± 2.07 (11.51–19.95)	17.28 ± 2.28 (11.51–19.95)	17.17 ± 1.87 (13.78–19.85)
	56	29	27

sVEP Sweep Visual Evoked Potential, *GA* Gestational Age, *TSB*, Total Serum/Plasma Bilirubin, *cpd* Cycles Per Degree.
*Student's unpaired t test; †χ^2 test.
∂TSB thresholds for treatment of hyperbilirubinemia was assessed for infants < 34 weeks' gestation using Premie BiliRecs™;[15] for infants ≥ 34 weeks' gestation using the 2004 American Academy of Pediatrics Guideline[11] and 2009 clarification[12].

RESULTS

Patient demographics

154 preterm infants were enrolled. Supplementary Table 1 shows the demographics and whether or not infants were treated for hyperbilirubinemia, provided for the entire cohort, and also stratified by prematurity. GA, birthweight, and TSB levels were significantly different based on prematurity.

Demographics and degree of hyperbilirubinemia of the 57/154 enrolled (37%) who completed their follow-up sVEP examinations and vision assessments are shown in Table 1. Because there were only 2 infants < 28 weeks' gestation, they were combined with those aged 28 to < 34 weeks, to yield 2 preterm cohorts: <34 weeks ($n = 29$; GA 31.1 ± 1.9 weeks; birthweight 1553 ± 432 grams; males 48%) and 34 to < 37 weeks ($n = 28$; GA 34.7 ± 0.7 weeks; birthweight 2168 ± 315 grams; males 50%). GAs and birthweights between the 2 preterm cohorts were statistically different ($p < 0.001$).

TSB levels as a function of GA

When TSB levels were plotted as a function of GA for the entire study cohort ($n = 154$), they increased as GA increased (Fig. 1A)

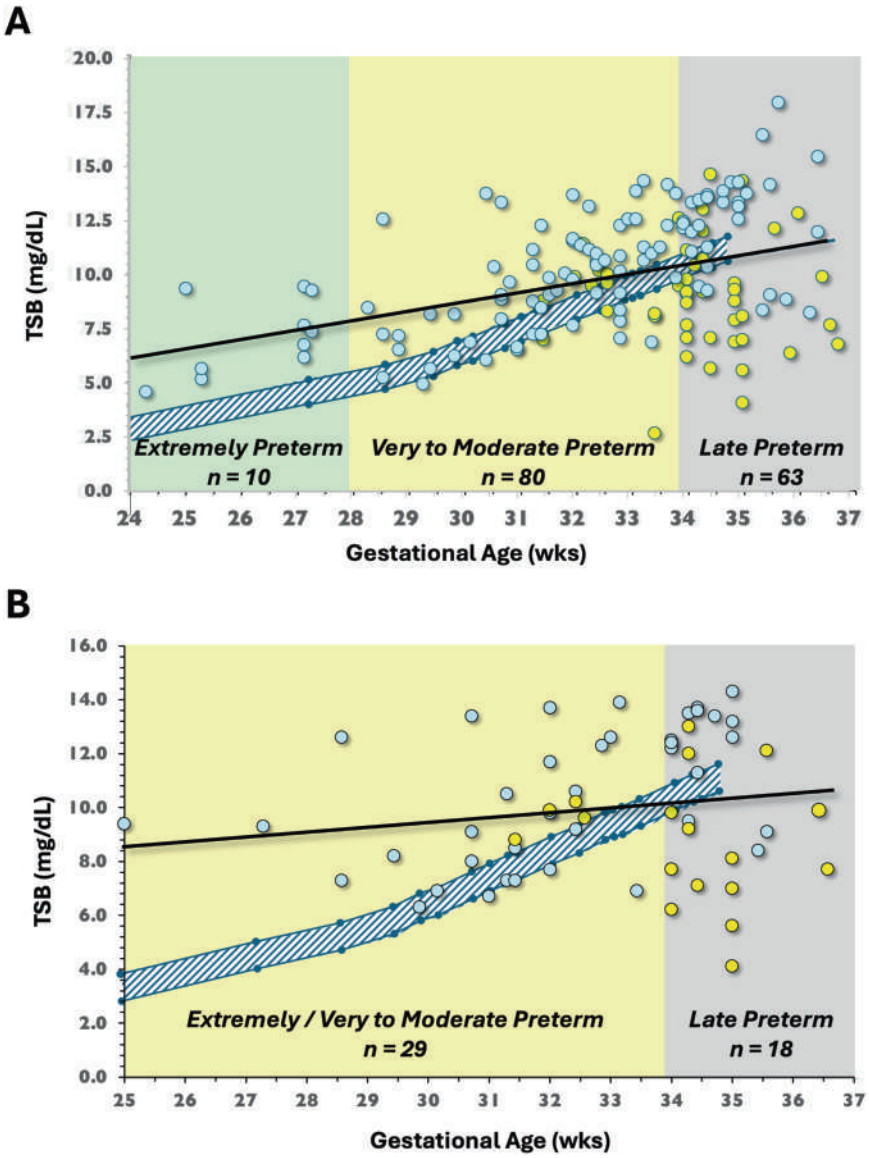

Fig. 1 TSB levels (mg/dL) as a function of GA (wks). A For our entire study cohort ($n = 153$), treated ($n = 107$, ◯) and untreated ($n = 46$, ◯) infants are shown. One infant born at 30.4 weeks' gestation had missing TSB data. **B** For the study cohort who were able to complete sVEP measurements at 12 months ($n = 57$), 39 infants were treated (◯) and 18 were untreated (◯). TSB thresholds for treatment of hyperbilirubinemia are shown by the hatched area as defined by Premie BiliRecs™ (<34 weeks) or the 2004 AAP Guideline / 2009 clarification (34 to < 37 weeks' gestation). Prematurity is designated by colored zones. Linear regression lines are shown in black.

with a Spearman correlation of 0.35 (95% CI 0.19–0.48; $p < 0.001$; slope 0.44; r^2 0.16; black line). Of these infants, 107 were treated (Fig. 1A, blue circles); whereas 46 (yellow circles) were untreated. TSB levels were measured at 3.9 ± 1.6 and 3.7 ± 1.3 postnatal days, respectively (Supplementary Table 1).

For the 57 infants who completed their follow-up sVEP examinations, TSB levels rose slightly as GA increased (Fig. 1B) with a Spearman correlation of 0.16 (95% CI –0.11–0.42; $p = 0.22$; slope 0.18; r^2 0.03; black line). 86% (25/29) of infants < 34 weeks' gestation were treated (TSB level of 9.6 ± 2.4 mg/dL); whereas, only 14% (4/29) were untreated (TSB level of 9.6 ± 0.6 mg/dL, $p =$ ns) (Table 2). In contrast, 50% (14/28) of infants 34 to < 37 weeks' gestation were treated (TSB level of 12.1 ± 1.9 mg/dL) and significantly higher than those untreated (8.5 ± 2.6 mg/dL; $p = 0.003$). Interestingly, the majority (78%, 14/18) of the untreated infants were late preterm infants.

Visual thresholds as a function of TSB levels and GA

Mean follow-up age was 11.6 ± 1.5 months (range: 8.8–14.6, target 12 months) with the majority (46/57) returning between 10 and 13 months (Table 1). No infant developed any stage of retinopathy of prematurity (ROP). Each sVEP metric was investigated for both GA and its confounder TSB. There was no correlation between Vernier acuity and GA (Fig. 2A, Spearman correlation of 0.0011 (95% CI -0.27–0.27; $p = 0.99$; slope –0.023; r^2 0.002; black line). However, Vernier acuity increased (i.e., worsened) as TSB levels increased for all infants (Fig. 2B, Spearman correlation of 0.46 (95% CI 0.32–0.58; $p < 0.001$; slope 0.26; r^2 0.31; black line). We then analyzed the data for the 2 preterm cohorts separately and noted a greater worsening of Vernier acuity in those infants < 34 weeks' GA (Fig. 2B, Spearman correlation of 0.78 (95% CI 0.57–0.89; $p < 0.001$; slope 0.47; r^2 0.49; blue line) than for those aged 34 to <37 weeks' gestation (Spearman correlation of 0.55 (95% CI 0.21–0.77; $p = 0.003$; slope 0.16 ($p = 0.003$); r^2 0.32; red line). The

Table 2. Patient demographics of infants who completed sVEP exams at 12 months of age ($n = 57$) stratified by each preterm cohort and having been treated or untreated.

Treated†	All ($n = 57$)		< 34 weeks ($n = 29$)		34 to < 37 weeks ($n = 28$)	
	Yes	No	Yes	No	Yes	No
n	39 (68%)	18 (32%)	25 (86%)	4 (14%)	14 (50%)	14 (50%)
GA (weeks)	32.2 ± 2.4 (25.0–35.6)	34.2 ± 1.4 (31.4–36.6)	30.9 ± 2.0 (25.0–33.4)	32.1 ± 0.5 (31.4–32.6)	34.6 ± 0.5 (34.0–35.6)	34.8 ± 0.8 (34.0–36.6)
			*$p = 0.02$			
Birthweight (grams)	1780 ± 489 (870–2745)	2019 ± 453 (700–2740)	1544 ± 411 (870–2363)	1608 ± 617 (700–2080)	2200 ± 301 (1750–2745)	2136 ± 337 (1660–2740)
Sex						
Male	22 (56%)	6 (33%)	13 (52%)	1 (25%)	9 (64%)	5 (36%)
Female	17 (44%)	12 (67%)	12 (48%)	3 (75%)	5 (36%)	9 (64%)
TSB (mg/dL)	10.5 ± 2.5 (6.3–14.3)	8.8 ± 2.3 (4.1–13.0)	9.6 ± 2.4 (6.3–13.9)	9.6 ± 0.6 (8.8–10.2)	12.1 ± 1.9 (8.4–14.3)	8.5 ± 2.6 (4.1–13.0)
	*$p < 0.02$		not significant		*$p < 0.001$	
Postnatal Age (days)	3.9 ± 1.6 (2.0–9.0)	3.6 ± 1.3 (2.0–6.0)	3.9 ± 1.8 (2.0–9.0)	3.0 ± 0.8 (2.0–4.0)	3.8 ± 1.2 (2.0–7.0)	3.8 ± 1.4 (2.0–6.0)
Age at 12-month Visit (months)	11.5 ± 1.5 (8.8–14.6)	11.9 ± 1.4 (9.7–14.3)	11.2 ± 1.3 (8.8–14.5)	11.6 ± 1.0 (10.5–12.5)	12.2 ± 1.7 (9.4–14.6)	11.9 ± 1.5 (9.7–14.3)
	39	18	25	4	14	14
Vernier Acuity (arcmin)	2.29 ± 1.21 (0.82–5.85)	1.90 ± 1.22 (0.72–5.86)	2.24 ± 1.44 (0.82–5.85)	3.07 ± 1.89 (1.77–5.86)	2.37 ± 0.66 (1.46–3.44)	1.57 ± 0.76 (0.72–3.10)
	39	18	25	4	14	14
					*$p = 0.01$	
Contrast Sensitivity (%)	2.04 ± 1.02 (0.63–4.40)	1.66 ± 0.68 (0.81–2.93)	1.84 ± 0.91 (0.63–4.32)	1.75 ± 0.71 (1.00–2.55)	2.41 ± 1.15 (1.20–4.40)	1.63 ± 0.70 (0.81–2.93)
	38	18	25	4	13	14
					*$p = 0.046$	
Grating Acuity (cpd)	17.15 ± 2.22 (11.51–19.84)	17.37 ± 1.76 (14.13–19.95)	17.23 ± 2.38 (11.51–19.84)	17.61 ± 1.74 (15.76–19.95)	17.01 ± 1.98 (13.78–19.35)	17.31 ± 1.82 (14.13–19.85)
	38	18	25	4	13	14

sVEP Sweep Visual Evoked Potential, *GA* Gestational Age, *TSB* Total Serum/Plasma Bilirubin, *cpd*: Cycles Per Degree.
*Student's unpaired *t* test compared with infants having low hyperbilirubinemia within the preterm cohort.
†TSB thresholds for treatment of hyperbilirubinemia was assessed for infants < 34 weeks' gestation using Premie BiliRecs™;[15] for infants ≥ 34 weeks' gestation using the 2004 American Academy of Pediatrics Guideline[11] and 2009 clarification[12].

slope for infants < 34 weeks' gestation was significantly steeper than those 34 to < 37 weeks' gestation ($p = 0.003$) indicating a greater increase and suggestive of a "dose" effect of TSB levels on Vernier acuity in the younger preterm infants. Mean follow-up age was 11.2 ± 1.3 (range 8.8–14.5) and 12.0 ± 1.6 months (range 9.4–14.6) for infants < 34 and ≥ 34 to 37 weeks' gestation, respectively, $p = 0.04$. No difference in the mean follow-up age was observed between the preterm cohorts (Table 1). Although postnatal vision development occurs for all 3 vision functions, an analysis of whether this occurred and could have affected our results showed no effect of aging.

Furthermore, when infants were sub-stratified by those treated versus those untreated for each preterm cohort, worse Vernier acuity correlated with TSB in the treated infants < 34 weeks' gestation (Fig. 2C, Spearman correlation of 0.82 (95% CI −0.61–0.92; $p < 0.001$; slope 0.47; r^2 0.49; blue line), but not in those treated infants aged 34 to < 37 weeks' gestation (Spearman correlation of −0.046 (95% CI −0.58–0.51; $p = 0.88$; slope 0.011 ($p = 0.003$); r^2 0.001; red line). For the untreated group, neither preterm cohort showed correlations between Vernier acuity and TSB levels, possibly because of our low sample size (Fig. 2D). No difference in the mean follow-up age was observed between all preterm cohorts or between those infants treated or untreated (Table 2).

Similar to Vernier acuity, no association was observed between contrast sensitivity and GA (Fig. 3A, Spearman correlation of 0.033 (95% CI −0.24–0.30; $p = 0.81$; slope 0.009; r^2 0.0005; black line). As in the case of Vernier acuity, contrast sensitivity increased (i.e., worsened) as TSB levels increased for all infants (Fig. 3B, Spearman correlation of 0.63 (95% CI −0.44–0.77; $p < 0.001$; slope 0.21; r^2 0.35; black line). This relationship was also observed in the treated and untreated infants for both preterm cohorts (Fig. 3C, D).

However, there were no associations between grating acuity and GA (Supplementary Fig. 3A) or between grating acuity and TSB levels in any of the groups (Supplementary Fig. 3B) or those treated or untreated (Supplementary Fig. 3C, D).

DISCUSSION

We hypothesized that prematurity would exacerbate the effect of TSB levels on visuocortical functioning. In this study, we demonstrated that infants at approximately one year of corrected age, Vernier acuity and contrast sensitivity obtained by sVEP studies worsened as a function of TSB levels during their neonatal period: the higher the documented TSB, the worse were the two sVEP variables. Furthermore, in two preterm infants < 34 weeks' gestation, the sVEP variables were worse compared with other gestational age groups (Fig. 2C). Importantly, these findings are of

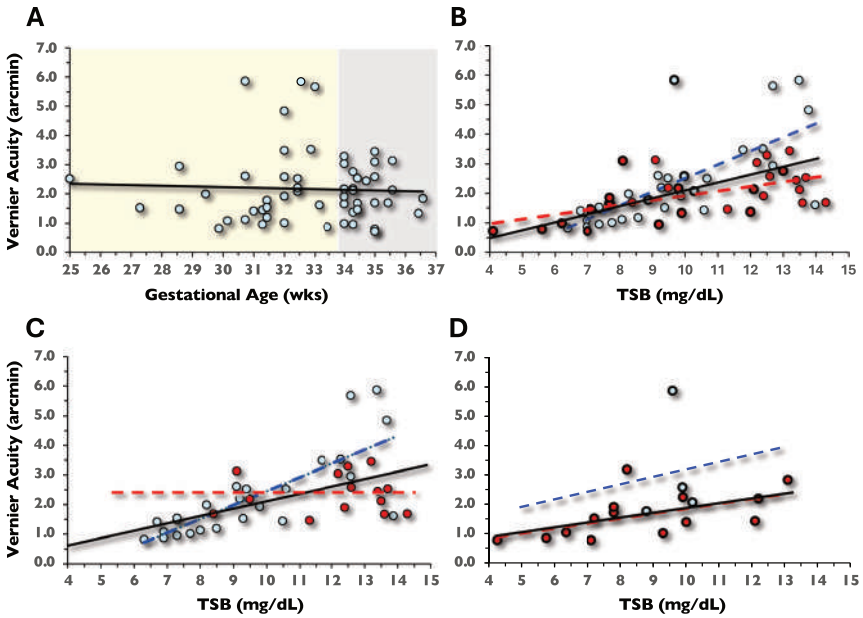

Fig. 2 Vernier acuity (arcsin). A as a function of GA (weeks) for infants who had sVEP measurements at 12 months ($n = 57$). Prematurity is designated by colored zones, linear regression lines are shown in black; and (**B**) as a function of TSB (mg/dL) for infants < 34 weeks' gestation (◯, $n = 29$, linear regression line is shown in blue dotted line) and 34 to < 37 weeks' gestation (●, $n = 28$, linear regression line is shown in red dotted line), those treated are shown with thick black borders. Treated (**C**), $n = 39$) and untreated (**D**), $n = 18$) in infants < 34 (◯ and ◉, linear regression lines are shown in blue dotted lines) and 34 to < 37 (● and ◉, linear regression lines are shown in red dotted lines) weeks' gestation.

concern since almost all infants received standard of care hyperbilirubinemia management. In fact, we did not observe extreme hyperbilirubinemia (TSB > 25 mg/dL), refractory response to phototherapy, need for exchange transfusion, nor symptoms of acute bilirubin encephalopathy. These observations stress that follow-up assessments of visual executive function in later childhood (5–7 years age) are warranted since visual development continues to occur for several years and being mostly developed by age 5. Most concerning is that a few preterm infants appear to be vulnerable to the negative effects on visual functioning despite their TSB levels not meeting thresholds for phototherapy use. We believe that visuocortical functioning is almost certainly harmed by excess TSB levels per se in these infants. Our observations are made even more evident on direct comparison with our previous study in term neonates with hyperbilirubinemia [3]. Term infants who we excluded in this study (since they were readmitted for hyperbilirubinemia) had higher TSB levels than those in our previous study and the worsening of Vernier acuity was more pronounced (Supplementary Fig. 4).

Clinical management of neonatal hyperbilirubinemia was exclusively guided in our study by the prevailing clinical recommendations and guidelines [11–13, 15–17]. Though it is well known that the TSB level is an imperfect index of bilirubin neurotoxicity [25], neither unbound bilirubin nor bilirubin-binding capacity measurements are used by any clinicians to manage neonates in United States intensive care nurseries. We designated the severity of hyperbilirubinemia (treated versus untreated) by the clinical discretionary administration of phototherapy. Phototherapy is the current standard of care and known to have no consequential adverse outcomes [26] and thus unlikely to affect sVEP measurements. The possibility of direct (local) retinal phototoxic injury is minimal because of widespread use of opaque shields to cover the infant eyes undergoing phototherapy.

Vulnerability to bilirubin neurotoxicity in both term and late-preterm infants with excess TSB levels and/or disordered albumin-binding is also affected by degrees of prematurity, hemolysis, perinatal-neonatal complications and individual infant vulnerability related to genetics, and social/economic and educational factors [12, 15]. The syndrome of bilirubin-induced neurologic disorders (BIND) has been proposed as a compendium of subtle clinical neurologic signs ranging from: i) neuromotor abnormalities; ii) muscle tone abnormalities; iii) hyper-excitable neonatal reflexes; iv) abnormal neurobehavior manifestations; v) speech and language abnormalities; and vi) display of central processing abnormalities such as sensorineural, audiological and visuomotor dysfunctions [8–10]. It has been hypothesized that bilirubin toxicity occurs at different thresholds depending on the brain region [27] and impacts the developmental mechanism active at the time of exposure [28]. Irreversible or transient post-icteric clinical sequelae have been reported to occur when TSB levels exceed an infant's neuroprotective defenses, resulting in neuronal injury to regions such as the basal ganglia, hippocampus, diencephalon, subthalamic nuclei, midbrain, cerebellum, and cerebellar vermis, and also to central and peripheral auditory and visual pathways, even at TSB thresholds lower than those usually associated with acute or chronic bilirubin encephalopathy [6]. These effects could be exacerbated by prematurity when the blood-brain barrier is less mature and thus more prone to bilirubin accumulating in the brain [29], and when immature neurons and glial cells are sensitive to bilirubin toxicity [30, 31]. Bilirubin can disrupt the maturation and myelination of oligodendrocytes, neurotransmission, synaptogenesis, and cell cycle arrest, and also interferes with postnatal brain development [10, 32–38]. Recent reports suggest that areas of the medulllo-pontine structures including those regulating oculomotor function are also affected [39]. Dose response of bilirubin neurotoxicity and initiation of a complex molecular pathological cascade is a subject of debate and scrutiny [7, 10, 39–41], but may be the underlying mechanism(s) responsible for our observed adverse postnatal visual changes. Whether these injuries are transient during infancy or permanent during late childhood has yet to be established.

Vernier acuity is a type of hyperacuity that utilizes extrastriate cortex to perceive fine line offsets (Supplementary Fig. 2) that are too small to be distinguished by retinal photoreceptor cells spacings, and therefore neural processing is invoked to offset

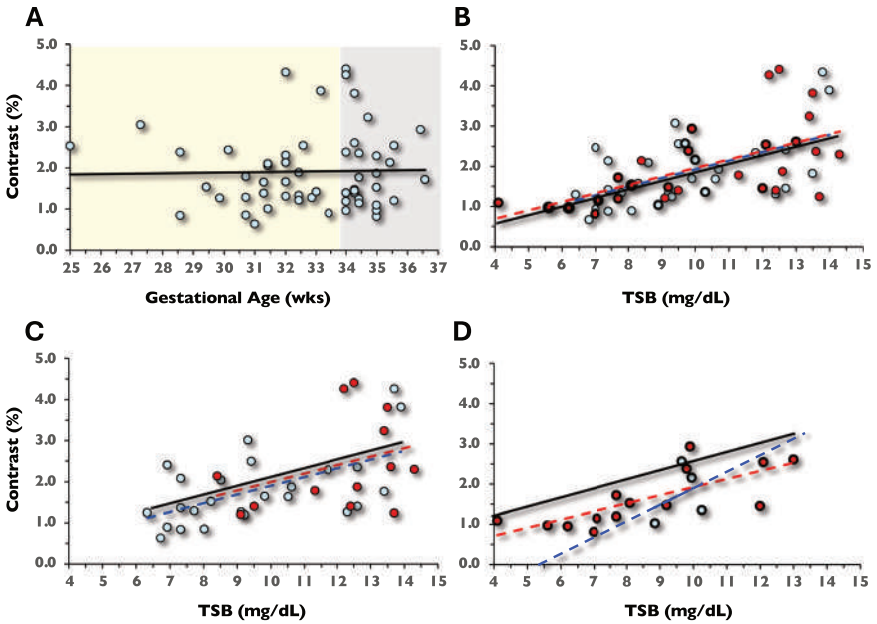

Fig. 3 Contrast sensitivity (%). A as a function of GA (weeks) for infants who had sVEP measurements at 12 months ($n = 56$). Prematurity is designated by colored zones; and (**B**) as a function of TSB (mg/dL) for infants < 34 weeks' gestation (◯, $n = 29$, linear regression line is shown in blue dotted line) and 34 to < 37 wks' gestation (●, $n = 28$, linear regression line is shown in red dotted line), those untreated are shown with thick black borders. Treated (**C**, $n = 39$) and untreated (**D**, $n = 18$) infants < 34 (◯ and ◯, linear regression lines are shown in blue dotted lines) and 34 to < 37 (● and ●, linear regression lines are shown in red dotted lines) weeks' gestation.

perception. The striate cortex processes information regarding lines and their orientation, while extrastriate cortex is involved in more complex tasks such as motion and form detection, and cerebral color vision [42, 43]. We found that Vernier acuity significantly worsened as a function of both GA and TSB levels, especially in those treated infants < 34 weeks' gestation (Fig. 2B, C). Early birth appears to add to the developmental burden of Vernier acuity.

Contrast sensitivity (Supplementary Fig. 2) often diminished by several conditions, including retinal disease (e.g., macular degeneration), optic nerve damage, aging, or cortical injury, also relies on extrastriate cortex for enhanced neural processing. We also observed a TSB-dependent worsening of contrast sensitivity (Fig. 3), but that however was not GA dependent unlike Vernier acuity. These findings suggest that extrastriate cortex is impacted by high TSB levels. Vernier acuity is strongly dependent on stimulus contrast [44] and thus contrast sensitivity deficits in anterior visual pathways such as the striate cortex could contribute to an elevated (worsened) Vernier acuity. However, Vernier acuities were more elevated in the younger preterm infants, suggesting that an additional vulnerability (TSB or another perinatal risk factor). We observed that Vernier acuity and contrast sensitivity were correlated with TSB levels, suggesting a second hit in the cortex over and above a general effect of reduced contrast sensitivity.

In contrast, we observed no changes in grating acuity as a function of either GA or TSB levels. Grating acuity (Supplementary Fig. 2) is primarily encoded in the striate cortex and relatively less sensitive to damaging effects on the visual system [42, 45, 46]. The insensitivity and the location (striate cortex) of cortical stimulation likely explain these findings. Further inquiry of children with neonatal bilirubin exposure are required to understand the role of extrastriate cortex in higher order visual tasks and the pattern of loss of function (e.g., motion and form detection).

We have previously reported that compared with control infants, full-term Infants with hyperbilirubinemia had worse sVEP thresholds that correlated with TSB levels [4]. Prematurity alone was unlikely to have been the sole cause of increased susceptibility to vision-altering effects. In fact, a previous study demonstrated improved acuity of premature infants when compared with non-jaundiced full-term infants when all is normal [4]. The normal development of Vernier acuity is most pronounced in the first few years but continues into adolescence. In this study, the postnatal age range at follow-up was 9–15 months and we did not detect any measurable improvements. Had we tested at a younger age (e.g., at 9-months corrected age) worse visuocortical dysfunction may have been observed. Moreover, the lack of postnatal improvement in visual development (acuity change) is of concern and suggests lasting deleterious effects on Vernier acuity and contrast sensitivity thresholds. Since our assessments were performed at a mean postnatal age of 11.6 months (similar to that in our full-term infant study of 12 months) [3] and well past the time of bilirubin exposure, visuocortical damage may be potentially long-lasting.

Our study does have some limitations. Infants with hyperbilirubinemia could not be ethically randomized to receive phototherapy or not due to current standard of care and risks of bilirubin neurotoxicity. Although the interlaboratory variability of TSB measurements between the two study institutions was a concern, differences were minimized at both sites by using identical instrumentation and standards. In addition, the etiologies of hyperbilirubinemia were not assessed. Potential bilirubin toxicity was not biochemically determined since routine clinical use of albumin-bilirubin binding, bilirubin-binding capacity, or unbound bilirubin are not practiced. Finally, our low follow-up rate (57/154) for sVEP exams was due to parental time constraints (each exam is approximately 2 h, cost/time of travel, and childcare issues) as well as infant illness and COVID-19 exposure concerns contributed to the small sample size in our sVEP findings.

CONCLUSIONS

In summary, we report long-term bilirubin-induced impaired functioning of the visual cortex in preterm neonates with dose-dependent hyperbilirubinemia. These findings underscore that the current guidelines to manage hyperbilirubinemia in preterm infants may not sufficiently or accurately identify those at risk for bilirubin neurotoxicity and that some infants with low (or

untreated) hyperbilirubinemia probably could have benefited from phototherapy.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

WVG—Conceptualized and designed the study, drafted the initial manuscript, and critically reviewed and revised the manuscript. RJW—Conceptualized and designed the study, drafted the initial manuscript, and critically reviewed and revised the manuscript. CH—Conceptualized and designed the study, drafted the initial manuscript, and critically reviewed and revised the manuscript. VKB—Conceptualized and designed the study, drafted the initial manuscript, and critically reviewed and revised the manuscript. AMN—Contributed and critically reviewed all data analysis and reviewed and revised the manuscript for important intellectual content. DKS—Contributed and critically reviewed all data analysis and reviewed and revised the manuscript for important intellectual content. MQM—Coordinated and supervised the collection of data, reviewed all data, and critically reviewed and revised the manuscript. JC—Collected data, carried out the initial analyses, and critically reviewed and revised the manuscript. AW-K-Y—Recruited and enrolled all study patients, coordinated and supervised data collection, and critically reviewed and revised the manuscript. GAM—Recruited and enrolled all study patients,

coordinated and supervised data collection, and critically reviewed and revised the manuscript. DR—Recruited and enrolled all study patients, coordinated and supervised data collection, and critically reviewed and revised the manuscript. CL—Recruited and enrolled all study patients, coordinated and supervised data collection, and critically reviewed and revised the manuscript. JSL—Recruited and enrolled all study patients, coordinated and supervised data collection, and critically reviewed and revised the manuscript. LD—Recruited and enrolled all study patients, coordinated and supervised data collection, and critically reviewed and revised the manuscript. TS—Recruited and enrolled all study patients, coordinated and supervised data collection, and critically reviewed and revised the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the institutional review board at Sutter Health and The Smith-Kettlewell Eye Research Institute. Signed parental informed consents were obtained. It was determined that waiver of information consent may be granted for this project as it meets requirements outlined in 45 CFR 46.116(f). All methods were performed in accordance with relevant guidelines and regulations. The study was performed in accordance with the Declaration of Helsinki.

ADDITIONAL INFORMATION

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