INTRODUCTION

Burdjalov et al. developed systematic amplitude-integrated EEG (aEEG) interpretation guidelines for the term and preterm neonatal population. This approach uses a subjective review of the aEEG trace to determine the continuity of cerebral activity (i.e. the proportion of activity above a set amplitude threshold), the presence or absence of sleep–wake cycling (SWC) and the minimum amplitude or ‘baseline’ of the trace, as well as the difference between the minimum and maximum amplitudes. In addition, other work has demonstrated that there is a clear qualitative pattern of maturation in the aEEG traces of premature infants without neurodevelopmental impairment, which parallels that of their overall development and this analysis can also be used to predict outcomes.

Digitally recorded aEEG traces allow for computation of a broad array of signal characteristics that can be readily compared across a cohort. The simplest measures, minimum, maximum and mean amplitude, allow for assessment of continuity. Fast Fourier transformation, which decomposes a complex signal into individual frequencies, enables a more granular view into the exact distribution of activity within a defined frequency band. Pattern recognition algorithms can identify characteristic electrographic signals that evolve in parallel with overall development, such as the length of time between bursts (the interburst interval or IBI) or specific wave patterns such as delta waves, whose appearance in a neonatal aEEG is developmentally regulated. The value of an automated quantitative approach to aEEG interpretation has been discussed in several different forums and has been applied largely to automated seizure detection algorithms with some remarkable success.

A remaining challenge, which must be overcome for widespread adoption of quantitative aEEG techniques, is the development of a ‘normative’ data set for comparison. Thornberg and Thiringer published one of the earliest data sets; however, they did not record any infants born before 30 weeks estimated gestational age, had no long-term neurodevelopmental data and reported only the minimum and maximum voltages. Natalucci et al. published data demonstrating predictable changes in amplitude characteristics over a broader range of gestational ages and included term-equivalent imaging as the measure of outcome. Nevertheless, these data are confounded by collection only during the first 96 h of life, a limited number of quantitative measures and no long-term neurodevelopmental data. Niemarkt et al. described an even broader array of amplitude and spectral...
characteristics, which was collected longitudinally in a cohort of 18 preterm infants who subsequently had developmental testing scores within the normal range at 1 and 2 years of corrected age (CA), respectively. However, there is emerging concern that neurodevelopmental testing at 2 years of age does not accurately predict outcomes at school age or beyond, and no further follow-up data are available.

To address these issues, we designed a study that outlines a full array of quantitative aEEG characteristics, including amplitude, spectral and wave pattern features, in a carefully selected population of preterm infants with favorable neuroimaging at term-equivalent age and neurodevelopmental outcomes at 2 and 7 years of age. We aim to establish a ‘normative’ range of aEEG measures for this population and hypothesize that healthy preterm infants will demonstrate a predictable pattern of maturation evident by quantitative analysis.

METHODS

Subjects

The subjects in this analysis are a carefully chosen subset of a larger cohort recruited in a prospective monitoring and imaging study conducted by the Victorian Infant Brain Study (VIBeS) group at the Murdoch Children’s Research Institute in Victoria, Australia. Subjects were premature infants born at $\leq 30$ completed weeks of gestation and admitted to the Royal Women's Hospital in Melbourne, Australia between July 2001 and December 2003. Parents of eligible infants were approached either before delivery or as soon as possible after delivery for informed consent.

Inclusion criteria

Other aspects of this cohort have been described elsewhere in the literature. For the purposes of this study, subjects were included in the analysis if they were determined to meet strictly defined outcome parameters in terms of both radiographic injury and subsequent neurodevelopmental testing. Favorable injury outcome was defined as no greater than grade 1 IVH on cranial ultrasound and no greater than mild white matter injury on term equivalent MRI. Favorable developmental outcome was assessed at two age points, 2 years (Bayley Scales of Infant Development, 2nd Edition (The Psychological Corporation, San Antonio, TX, USA) with both mental developmental index and psychomotor developmental index subscores $\geq 85$) and at 7 years (Wechsler Abbreviated Scale of Intelligence (The Psychological Corporation, San Antonio, TX, USA) with a score $\geq 85$).

Study design

Information regarding the sex, gestational age, birth weight and Apgar scores were obtained from the subject’s medical chart. Hydrospot electrodes (Physiometrix, North Billerica, MA, USA) were applied in the conventional C3-P3, C4-P4 configuration and traces were recorded using the BRM2 monitor (Natus Medical, San Carlos, CA, USA). Each recording was a minimum of 120 min in length and was conducted on days 4, 7, 14 and 28 of life, regardless of gestational age at birth. All quantitative analysis was performed using Analyze (Natus Medical), an offline aEEG review software package.

Cranial ultrasounds were obtained four times during the hospital course: during the first 72 h, days 7 to 10, day 28 and before discharge as per routine clinical practice. All infants underwent an MRI scan at term equivalent age without sedation. Infants were fed, swaddled, outfitted with earmuffs and placed in a vacuum fixation beanbag. Sleeping infants were scanned in a 1.5 T Signa LX Echospeed MRI System (General Electric, Milwaukee, WI, USA).

Basic analysis

The recorded aEEG data were visually inspected, and regions of the recording with electrode impedance exceeding 15 kΩ were discarded. The middle 60 min of each 120 min recording was used for analysis, with the aim of avoiding artifacts sometimes found at the beginning and end of aEEG recordings. This 1-h segment was then visually reviewed to ensure that it was free of artifacts and seizures. The minimum, maximum and mean amplitude of each trace was then calculated. A sample tracing with quantitative measures highlighted is shown in panel 1 of Figure 1.

**Figure 1.** Panel 1 depicts a 90 min amplitude-integrated electroencephalography (aEEG) recording. Line A represents the median maximum value (12.3 μV), line B the mean value (8.8 μV) and line C the median minimum value (4.3 μV). Panel 2 depicts the four amplitude bands that were assessed to determine the content of the trace. Band A represents those with a mean value $> 100$ μV, Band B represents those with a mean value $> 50$ μV and Band C those with a mean value $> 25$ μV. Band D those $> 100$ μV.

© 2015 Nature America, Inc.
Continuity
Hellström-Westas et al.² standardized the terminology used to describe aEEG backgrounds with a five-category system consisting of continuous normal voltage, discontinuous normal voltage, continuous low voltage, burst suppression and flat tracing. Each category is defined by minimum and maximum voltage parameters. Continuity can be evaluated quantitatively by determining the average baseline of a trace as compared with a predetermined threshold¹⁹ or by determining the percentage of the trace contained within certain voltage thresholds.²⁰ In this data set, the percentage of 2-s intervals where the amplitude of the recorded signal was maximum IBI.

Sleep–wake cycling
The trace was inspected for evidence of SWC, defined as rhythmic sinusoidal elevations of the baseline lasting longer than 20 min.²¹ The SWC pattern was deemed ‘mature’ if it was the predominant pattern during the recording period, ‘intermediate’ if cycling was present but not predominant and ‘absent’ if SWC was not present.

Spectral edge frequency analysis
Spectral edge frequency (SEF) has been used by researchers to determine the frequency distribution in aEEG traces in healthy¹⁴,²⁰ and injured²² preterm infants. Fast Fourier transformation was used to determine SEF 90, defined as the frequency between 2 and 20 Hz, below which 90% of the power was present in the same 60-min epoch.

Bayesian probability analysis
A software algorithm developed by Mitchell et al.²³ using a broader array of the same infants in this cohort, takes a Bayesian inference approach to identify delta waves (including separate components of smooth delta waves and delta brushes) and theta bursts. In addition, it can calculate the percentage of the trace that is spent in the IBI and the length of the SWC was noted in increasing proportions as PMA advanced, similar in proportion to that noted in prior studies.²¹ An overview is provided in Table 4.

Delta and theta activity
A significant negative correlation was noted with increasing PMA and counts of all wave types including smooth delta wave

### Table 1. Sample perinatal and clinical characteristics

| Characteristic                                      | n = 18 |
|-----------------------------------------------------|--------|
| Gestational age at birth, m ± s.d. (weeks)          | 27.3 ± 1.9 |
| Male, n (%)                                         | 9 (50) |
| Birth weight, m ± s.d. (g)                          | 976 ± 173 |
| Intraventricular hemorrhage                         |        |
| None, n (%)                                         | 16 (89) |
| Grade 1, n (%)                                      | 2 (11)  |
| White matter injury                                 |        |
| None, n (%)                                         | 10 (56) |
| Mild non-cystic, n (%)                              | 8 (44)  |
| Need for inotropic agents, n (%)                   | 4 (22)  |
| Need for sedative agents, n (%)                    | 0 (0)   |
| Antenatal steroids, n (%)                           | 17 (94) |
| Culture-proven sepsis, n (%)                       | 3 (17)  |
| BPD², n (%)                                         | 6 (33)  |
| BPD-II MDI, m ± s.d.                                | 98 ± 9  |
| PDI, m ± s.d.                                       | 99 ± 9  |
| WASI FS IQ, m ± s.d.                                | 104 ± 15 |

Abbreviations: FSIQ, Full-Scale IQ; m, mean; MDI, mental development index; PDI, psychomotor development index; PMA, postmenstrual age; WASI, Wechsler Abbreviated Scale of Intelligence. *Defined as need for supplemental oxygen at 36 weeks PMA.
(R = −0.39, P = 0.001), delta brush (R = −0.37, P = 0.003) and theta burst (R = −0.61, P = 5.66 × 10^{-3}). Median detected wave counts for each gestational age grouping are provided in Table 5.

Interburst interval
The mean percentage of the recording spent in the IBI decreased as PMA increased, with a significant negative correlation (R = −0.38, P = 0.001). The maximum length of the IBI decreased with increasing PMA, with a significant negative correlation (R = −0.27, P = 0.03). Complete information about changes in the IBI at different PMAs is provided in Table 5.

**DISCUSSION**
A significant limitation preventing the widespread adoption of routine EEG monitoring in neonatal intensive care units is the need for interpretation by skilled epileptologists, adding a significant time and cost burden to health-care systems. aEEG has filled this gap in many centers around the world, using pattern recognition to provide information about brain function that can be readily interpreted by non-expert clinicians, enabling detection of electrographic seizures and assessment of background activity. Nevertheless, both conventional and aEEG methods rely on subjective interpretation, which can be variable, time consuming and requires a degree of experience.

This study uses quantitative methods on aEEG to confirm the maturational patterns that have been previously described in the literature using qualitative analysis. Our analysis of the content of these aEEG recordings supports a predictable transition that is strongly correlated with PMA. This developmental continuum

### Table 2. Comparison of perinatal factors between included and excluded subjects

| Gestational age at birth, m ± s.d. (weeks) | Included (n = 18) | Excluded (n = 206) | P-value |
|-------------------------------------------|------------------|-------------------|---------|
| Male, n (%)                               | 9 (50)           | 105 (51)          | 0.992   |
| Birth weight, m ± s.d. (g)                | 976 ± 173        | 927.6 ± 197       | 0.308   |
| Antenatal steroids, n (%)                 | 17 (94%)         | 181 (88%)         | 0.260   |
| Intrauterine growth restriction, n (%)    | 2 (11)           | 16 (8)            | 0.937   |

**Abbreviation:** m, mean.

### Table 3. Amplitude characteristics by PMA

| Group | < 27 weeks (n = 8) | 27-29 weeks (n = 16) | 29-31 weeks (n = 21) | > 31 weeks (n = 19) |
|-------|-------------------|----------------------|----------------------|---------------------|
| Min amplitude, M (range) | 1.7 (1.3–2.8) | 2.1 (1.5–2.8) | 2.4 (1.1–3.7) | 3.1 (2.0–3.5) |
| Mean amplitude, M (range) | 6.2 (5.6–8.6) | 5.8 (4.4–8.3) | 6.2 (3.9–8.4) | 6.3 (4.7–8.8) |
| Max amplitude, M (range) | 12.9 (8.6–15.7) | 10.7 (5.1–15.9) | 10.4 (5.4–15.7) | 9.7 (5.5–15.5) |
| SEF 95, M (range) | 7.9 (7.4–8.8) | 8.3 (7.3–9.5) | 9.4 (8.6–10.7) | 10.1 (9.0–11.2) |
| > 10 μV, M (range) | 100 (97–100) | 100 (94–100) | 100 (88–100) | 100 (100–100) |
| > 25 μV, M (range) | 76 (64–89) | 78 (55–89) | 80 (56–93) | 87 (71–100) |
| > 50 μV, M (range) | 46 (40–59) | 44 (30–64) | 47 (24–60) | 39 (13–69) |
| > 100 μV, M (range) | 22 (16–29) | 17 (4–28) | 12 (1–23) | 5 (0–16) |

**Abbreviations:** M, median value; PMA, postmenstrual age; SEF, spectral edge frequency. *Percentage of 1-min epochs with average baseline exceeding given threshold.

### Table 4. Sleep–wake cycling by PMA

| Group | < 27 weeks (n = 8) | 27-29 weeks (n = 16) | 29-31 weeks (n = 21) | > 31 weeks (n = 19) |
|-------|-------------------|----------------------|----------------------|---------------------|
| SWC absent | 2 | 2 | 0 | 0 |
| SWC intermediate | 6 | 14 | 16 | 6 |
| SWC mature | 0 | 0 | 5 | 13 |

**Abbreviations:** PMA, postmenstrual age; SWC, sleep–wake cycling.

### Table 5. Wave characteristics by PMA

| Group | < 27 weeks (n = 8) | 27-29 weeks (n = 16) | 29-31 weeks (n = 21) | > 31 weeks (n = 19) |
|-------|-------------------|----------------------|----------------------|---------------------|
| SDW, M (range) | 191 (112–315) | 167 (57–367) | 131 (24–350) | 101 (18–254) |
| DB, M (range) | 61 (25–74) | 47 (5–94) | 34 (0–119) | 20 (3–70) |
| TB, M (range) | 21 (9–36) | 7 (0–41) | 1 (0–9) | 1 (0–6) |
| IBI* %, m (s.d.) | 38 (14) | 35 (9) | 33 (12) | 24 (9) |
| Max. IBI length (s), m (s.d.) | 31 (17) | 41 (51) | 24 (10) | 17 (8) |

**Abbreviations:** DB, delta brush; m, mean; M, median; PMA, postmenstrual age; SDW, smooth delta wave; TB, theta burst. *Percentage of recording time between bursts.
transitions from the infrequent high amplitude bursts and long low-voltage quiescent periods of burst suppression and tracé discontinue, which predominate the earliest gestational ages, to the continuous, moderate voltage activity with infrequent quiet periods of tracé alternans associated with more developmentally mature infants.

These findings have significant biological relevance as there is compelling evidence that early cortical activity is important for brain growth, the absence or delay of which can be predicted by a persistent or transient arrest in electrophoretic maturation and is known to be associated with adverse neurodevelopment.

More specifically, an increased IBI (and thus overall decreased cortical activity) has been associated with adverse events both in the acute setting such as acidosis or sedative administration as morphine and phenobarbital, as well as long term with morphine and phenobarbital,

as well as with increased likelihood of developmental handicap at 2 years CA.

Technical aspects of aEEG signal processing, as is the case with all quantitative aEEG or EEG analysis, limit this study. To produce the aEEG output, the raw signal is transformed, first by an asymmetric bandpass filter, which attenuates the signal between 2 and 15 Hz, followed by rectification and averaging over a moving 2-s time window. This design, while intentionally minimizing the unwanted signals introduced by muscle artifact, also removes the low-frequency component of cerebral activity. As a result, the aEEG signal is not a 1:1 representation of a traditionally acquired EEG trace, nor is it intended to be. Nevertheless, the correlation between these quantitatively determined features and PMA is predictable, reproducible and is remarkably similar to similar examinations made using conventional EEG.

Constructing a longitudinal cohort of former preterm infants with development outcomes indistinguishable from the general population is quite challenging. Every step along the way carries the potential risk of exclusion, largely because of death or disability. Indeed, given the NICHD Neonatal Research Network data, only 44% of the infants in this study’s cohort, with a mean estimated gestational age of 27 weeks, would be expected to survive without major morbidity at the time of hospital discharge, and much less have a neurodevelopmental outcome within the normal range at school age. As described earlier, Niemarkt et al. constructed a similar longitudinal cohort, which examined 4% (18/449) of the initially eligible infants, very similar to the 5% (18/348) discussed in this study, particularly given our collection of an additional time point of data at age 7 years. These additional data proved quite valuable, as a further testing revealed that 10% of the cohort that appeared to be developing normally at 2 years CA had fallen behind by school age.

The results of this study have both clinical and research applications. Although this analysis is presented as group data, all 18 infants exhibited the same developmental maturation patterns along a similar trajectory. This reproducibility allows clinicians to use this information as an aid in identifying preterm infants with reassuring aEEG parameters, potentially assisting in the management of the patient or counseling the parents of pre-mature infants. Similarly, these same parameters can be longitudinally tracked in future prospective studies to assess the potential impact of a number of frequently encountered clinical situations that have been associated with adverse neurodevelopmental outcomes such as sepsis, necrotizing enterocolitis and medications such as morphine, dexmethyladine and general anesthesia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Peter Anderson, PhD, and the Victorian Infants Brain Study research group for their assistance in collection and analysis of the presented data. This work was supported by National Institutes of Health, NICHD (P30 HD062171 and R01 HD057098) and Doris Duke Distinguished Clinical Scientist Award.

REFERENCES

1. Burdjlavov VF, Baumgart S, Spitzer AR. Cerebral function monitoring: a new scoring system for the evaluation of brain maturation in neonates. Pediatrics 2003; 112(4): 855–861.
2. Hellström-Westas L, De Vries LS, Rosen I. Atlas of Amplitude-Integrated EEGs in the newborn. Informa Healthcare; Distributed in North and South America by Taylor & Francis: London; Boca Raton, FL, 2008.
3. Vecchieri M-F, André M, d’Allest AM. Normal EEG of premature infants born between 24 and 30 weeks gestational age: terminology, definitions and maturation aspects. Neuropediatrics Clin Neurophysiol 2007; 37(5): 311–323.
4. West CR, Harding JE, Williams CE, Nolan M, Battin MR. Cot-side electroencephalography for outcome prediction in preterm infants: observational study. Arch Dis Child Fetal Neonatal Ed 2011; 96(2): F108–F113.
5. Niemarkt HJ, Andriessen P, Peters CHL, Pasman JW, Blanco CE, Zimmermann LJ, et al. Quantitative analysis of amplitude-integrated electroencephalogram patterns in stable preterm infants, with normal neurological development at one year. Neonatology 2010; 97(2): 175–182.
6. Palmu K, Wikström S, Hippeläinen E, Boylan G, Hellström-Westas L, Vanhatalo S. Detection of ‘EEG bursts’ in the early preterm EEG: visual vs automated detection. Clin Neurophysiol 2010; 121(7): 1015–1022.
7. Wilson SB. A neural network method for automatic and incremental learning applied to patient-dependent seizure detection. Clin Neurophysiol 2005; 116(8): 1785–1795.
8. Roessgen M, Zoubir AM, Boashash B. Seizure detection of newborn EEG using a model-based approach. IEEE Trans Biomed Eng 1998; 45(6): 673–685.
9. Liu A, Hahn JS, Heldt GP, Coen RW. Detection of neonatal seizures through computerized EEG analysis. Electroencephalogr Clin Neurophysiol 1992; 82(1): 30–37.
10. Gotman J, Flanagan D, Zhang J, Rosenberg B. Automatic seizure detection in the acquired EEG trace, nor is it intended to be. Nevertheless, the correlation between these quantitatively determined features and PMA is predictable, reproducible and is remarkably similar to similar examinations made using conventional EEG.

Constructing a longitudinal cohort of former preterm infants with development outcomes indistinguishable from the general population is quite challenging. Every step along the way carries the potential risk of exclusion, largely because of death or disability. Indeed, given the NICHD Neonatal Research Network data, only 44% of the infants in this study’s cohort, with a mean estimated gestational age of 27 weeks, would be expected to survive without major morbidity at the time of hospital discharge, and much less have a neurodevelopmental outcome within the normal range at school age. As described earlier, Niemarkt et al. constructed a similar longitudinal cohort, which examined 4% (18/449) of the initially eligible infants, very similar to the 5% (18/348) discussed in this study, particularly given our collection of an additional time point of data at age 7 years. These additional data proved quite valuable, as a further testing revealed that 10% of the cohort that appeared to be developing normally at 2 years CA had fallen behind by school age.

The results of this study have both clinical and research applications. Although this analysis is presented as group data, all 18 infants exhibited the same developmental maturation patterns along a similar trajectory. This reproducibility allows clinicians to use this information as an aid in identifying preterm infants with reassuring aEEG parameters, potentially assisting in the management of the patient or counseling the parents of pre-mature infants. Similarly, these same parameters can be longitudinally tracked in future prospective studies to assess the potential impact of a number of frequently encountered clinical situations that have been associated with adverse neurodevelopmental outcomes such as sepsis, necrotizing enterocolitis and medications such as morphine, dexmethyladine and general anesthesia.
of cerebral white matter injury in premature infants. Pediatrics 2003; 111(1): 27–33.

23 Mitchell TJ, Neil JJ, Zempel JM, Thio LL, Inder TE, Brethorst GL. Automating the analysis of EEG recordings from prematurely-born infants: a Bayesian approach. Clin Neurophysiol Off J Int Fed Clin Neurophysiol 2013; 124(3): 452–461.

24 Hellström-Westas L, Rosen I, de Vries LS, Greisen G. Amplitude-integrated EEG classification and interpretation in preterm and term infants. Neonatal Netw 2006; 25(2): e76–e87.

25 Benders MJ, Palmu K, Menache C, Borradori-Tolsa C, Lazeypas F, Sizonenko S, et al. Early brain activity relates to subsequent brain growth in premature infants. Cereb Cortex [Internet] 27 May 2014. Available at: http://www.cercor.oxfordjournals.org/cgi/doi/10.1093/cercor/bhu097 (7 November 2014)

26 Hayakawa F, Okumura A, Kato T, Kuno K, Watanabe K. Dysmature EEG pattern in EEGs of preterm infants with cognitive impairment: maturation arrest caused by prolonged mild CNS depression. Brain Dev 1997; 19(2): 122–125.

27 Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. Pediatrics 2014; 134(2): e444–e453.

28 Eaton DG, Wertheim D, Oozeer R, Dubowitz LM, Dubowitz V. Reversible changes in cerebral activity associated with acidosis in preterm neonates. Acta Paediatr 1994; 83(9): 486–492.

29 Bell AH, Greisen G, Pryds O. Comparison of the effects of phenobarbitone and morphine administration on EEG activity in preterm babies. Acta Paediatr 1993; 82(1): 35–39.

30 Wikström S, Ley D, Hansen-Pupp I, Rosén I, Hellström-Westas L. Early amplitude-integrated EEG correlates with cord TNF-alpha and brain injury in very preterm infants. Acta Paediatr 2008; 97(7): 915–919.

31 Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 2010; 126(3): 443–456.