Cortical motor activity modulates respiration and reduces apnoea in neonates

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Abstract

Respiration is governed by a widespread network of cortical and subcortical structures. This complex communication between the brain and lungs is altered in pathological conditions. Apnoea – the cessation of respiration – is a common condition in infants, particularly those born prematurely. Apnoea in infants is believed to relate to immaturity of brainstem respiratory centres; involvement of the cortex in respiration in infants has yet to be explored. We investigated if cortical control of respiration occurs in newborn humans and whether it relates to apnoea. Using simultaneous electroencephalography (EEG) and impedance pneumography, we show that cortico-respiratory coupling is present in premature and term newborns (104 recordings from 68 infants; 34.5 ± 2.6 weeks post-menstrual age), identifying an interplay between breathing phase and EEG amplitude. We further shed light on the biological meaning by revealing that the strongest coupling occurs during inspiration and that cortical activity precedes respiration, with coupling strongest over frontocentral regions. These findings support the notion that the cortico-respiratory coupling observed here primarily constitutes communication between cortical motor areas and lung effectors. Moreover, we show that cortico-respiratory coupling is negatively correlated with the rate of apnoea, revealing novel mechanistic insight into this common and potentially life-threatening neonatal pathology.

Introduction

Apnoea – the cessation of respiration – is a potentially life-threatening event[1], affecting more than 50% of preterm infants and some term infants[2]. Apnoeic episodes can result in physiological instability including oxygen desaturation and bradycardia[1], are associated with suppression of brain activity[3], and have been linked to poorer neurodevelopmental outcomes[4]. Apnoea of prematurity is caused by immaturity of the lungs, chest wall, and nervous system[5]. Immaturity of the brainstem plays a significant role in apnoea[1]; however, the relationship with other respiratory-linked brain regions is less clear. In adults with congenital central hypoventilation syndrome[6] or obstructive sleep apnoea[7], a respiratory-linked increase in cortical motor activity suggests that the motor cortex plays an important role in maintaining autonomous respiration.

Recently, it was proposed that communication between the cortex and lungs can be identified and quantified through so-called cortico-respiratory coupling[8,9]. This functional coupling arises when the amplitude of cortical activity is modulated by the respiration phase, or *vice versa*. This phase-amplitude coupling is typically quantified using non-invasive recordings capturing respiratory and neural activity (e.g., magneto- or electroencephalography [EEG]). Studies have demonstrated that the respiratory phase at low frequencies (<1 Hz) relates to higher-frequency oscillations in the cortex[9,10]. Phase-amplitude coupling therefore allows us to quantify the interplay between respiratory and cortical activity. In adults, this coupling can be observed in a wide network of subcortical and cortical regions including motor areas[9]. Moreover, cortico-respiratory coupling is aberrated in pathological conditions[11].

Here, using cortico-respiratory coupling, we investigated whether cortical motor areas play a role in respiration in infants (Figure 1). We hypothesised that the strength of cortico-respiratory coupling is negatively associated with apnoea rate. To this end, we first confirmed the existence of cortico-respiratory coupling in both premature and term infants. To investigate if the coupling relates to the central motor drive, we further studied the spatial localisation, timing throughout the respiratory cycle and coupling directionality. Finally, we examined the relationship between cortico-respiratory coupling and apnoea rate.

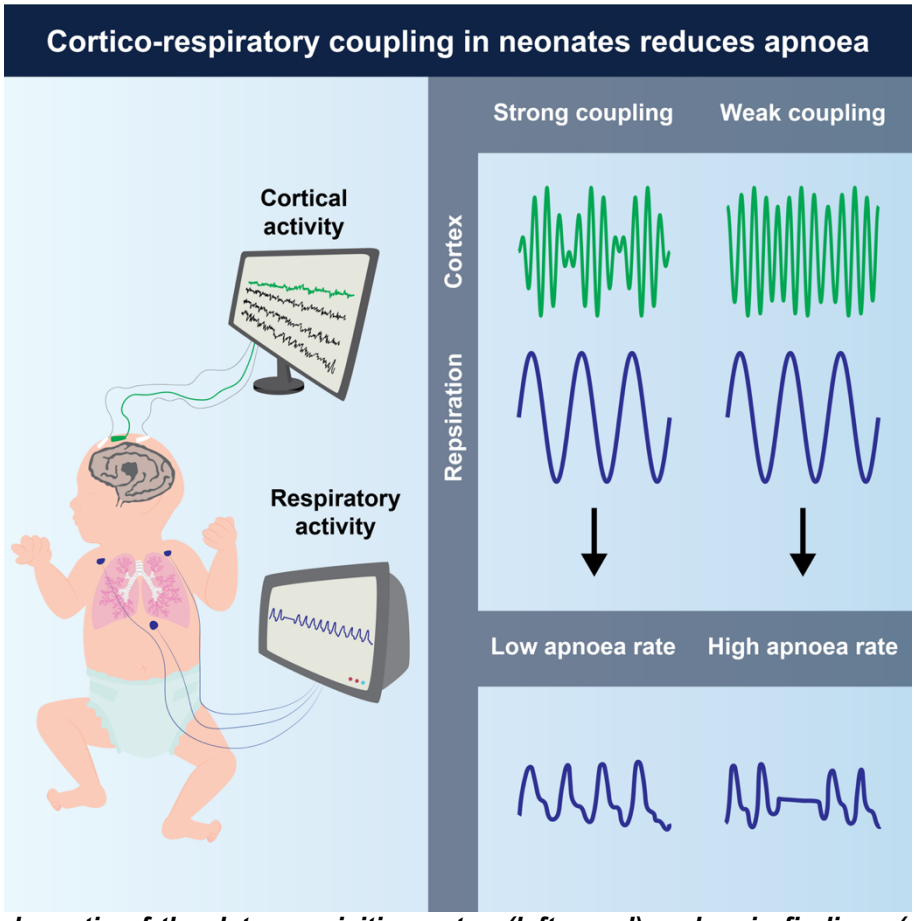

Figure 1. Schematic of the data acquisition setup (left panel) and main findings (right panel). *Each recording consisted of cortical activity (using electroencephalography) and respiratory activity (using impedance pneumography).*

Results and Discussion

Respiratory and cortical activity exhibit phase-amplitude coupling in infants

We analysed simultaneous 8-channel resting-state EEG (to measure cortical activity) and impedance pneumography (IP; to measure respiration) in 68 infants (28-42 weeks postmenstrual age [PMA] at time of recording) on 104 occasions. We first assessed whether and how EEG and IP are interrelated using cross-frequency coupling[9,12,13]. In line with findings in adults[9], we expected that the amplitude of oscillatory brain activity is modulated as a function of the respiratory cycle. That is, we expected to detect an EEG component that systematically varied with timing within the respiratory cycle. To this end, we identified individual breaths (and thus respiratory phase) from the IP signal[14]. Through averaging the EEG (time-locked to the IP signal) across breaths, we observed that EEG amplitude was modulated in the delta- (0.5-4 Hz) and theta-band (4-8 Hz) frequencies depending on the respiratory phase (Figure 2A-B). This analysis

can be seen as a first indication that the EEG amplitude in specific frequency ranges changes systematically with the respiratory phase.

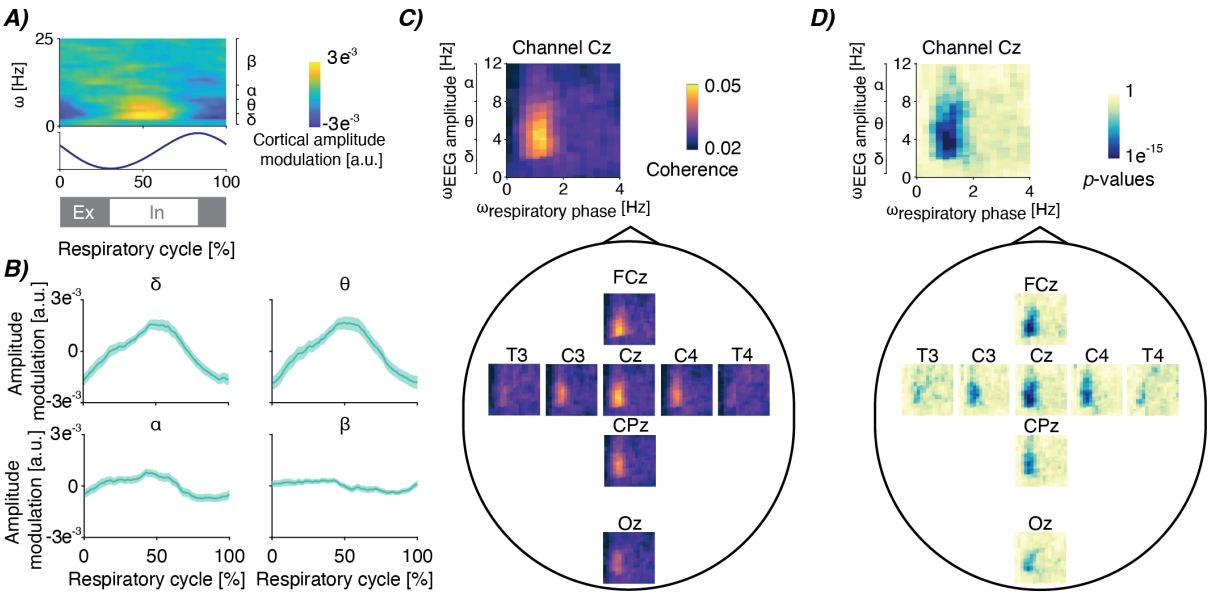

***Figure 2. Cortico-respiratory coupling in infants**. **A)** Electroencephalographic (EEG) amplitude modulation depending on the respiratory cycle and frequency. EEG amplitude is averaged over breaths, recordings and channels FCz and Cz. The blue graph below is the impedance pneumographic signal time-locked from breath to breath and averaged over breaths and recordings. Grey and white bars below indicate expiration (Ex; in grey) and inspiration (In; in white). **B)** The time-resolved amplitude modulations averaged within four frequency bands (delta: 0.5-4 Hz, theta: 4-8 Hz, alpha: 8-13 Hz, beta: 13-25 Hz), focusing on channels FCz and Cz again. Shaded light-green area is the standard error across recordings after pooling over channels. **C)** Spatial map of coherence-based phase-amplitude coupling (PAC) between the respiratory phase ($\omega_{respiratory\ phase}$) and EEG amplitude ($\omega_{EEG\ amplitude}$) – see Figure S1 for the pan-spectral PAC estimates. Spectra are the mean over all recordings. **D)** Spatial map for the corresponding statistical significance relative to surrogate PAC obtained through epoch shuffling (reported on a logarithmic scale).*

We then statistically confirmed this association between EEG and respiration using phase-amplitude coupling (PAC). Using cross-frequency coherence, we observed significant PAC between the phase at the mean respiratory frequency and the EEG amplitude in delta and theta frequency bands (Figure 2C-D). PAC was strongest at central electrodes (channels FCz and Cz; Figure 2C-D). In line with the results presented in Figure 2A, we did not find significant PAC at higher EEG frequencies such as the alpha (8-13 Hz) and beta (13-25 Hz) frequency bands (Figure S1).

These findings confirm that cortico-respiratory coupling occurs in infants. Whilst we are the first to show that cortico-respiratory coupling already exists in early life,

adult studies have revealed widespread brain-respiratory networks covering both cortical and subcortical areas, as well as pan-spectral components of oscillatory brain activity (2-150 Hz)[9]. These networks have been linked to a diverse range of perceptual, cognitive and motor processes[8,15-17]. In these studies, respiratory rhythm has been found to relate to task performance (e.g., better performances are found during a certain respiratory phase) as well as neural oscillations in the brain[11]. For example, during an isometric motor task, the strength of cortico-muscular communication at beta-band frequencies cyclically changes with the respiratory phase[16]. Moreover, the probability of initiating goal-directed movements relates to cortical motor activity (here, measured as the readiness potential amplitude) and respiratory phase[17]. These relationships between task performance, respiration and cortical activity are well-established and replicated in a variety of animal and human adult studies, demonstrating the importance of cortico-respiratory coupling.

Cortico-respiratory coupling is strongest during inspiration and driven by the cortex

To further shed light on the functional meaning of cortico-respiratory coupling in neonates, we next examined the timing and directionality of the coupling. To study whether coupling strength fluctuates throughout the respiratory cycle, we focused on the EEG channels with strongest coupling (FCz and Cz), and frequencies where statistically significant coupling occurs (Figure 2D; alpha level set at 0.001). We confirmed that PAC changes in a time-dependent manner, observing the strongest PAC during the inspiratory phase (Figure 3A-B). Such within-breath modulation is evident for both delta- and theta-frequencies (Figure 3A-B). This indicates that the strength of cortico-respiratory coupling changes throughout the respiratory cycle. During the inspiratory phase, neural activation from the central nervous system is required to drive respiratory motoneurons causing contraction of respiratory muscles. Given the phasic PAC modulation as seen in Figure 3A-B, this suggests that cortical areas centred around the vertex play an important role in the neural activation of respiratory muscles.

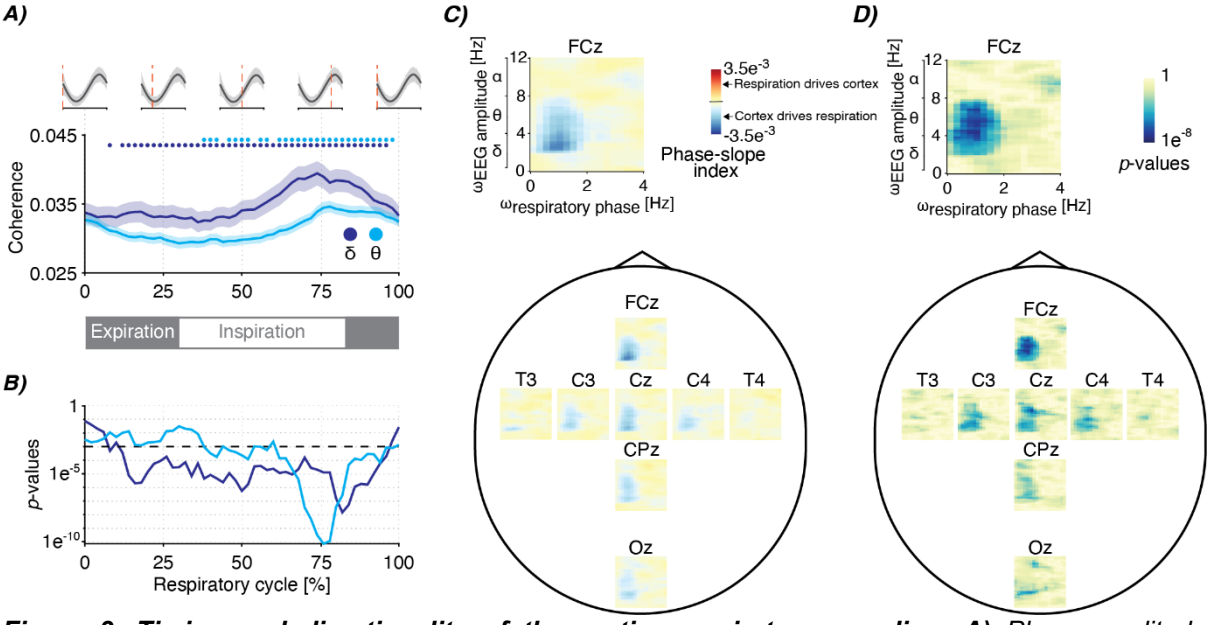

Figure 3. Timing and directionality of the cortico-respiratory coupling. A) *Phase-amplitude coupling modulation (estimated as cross-frequency coherence) throughout the respiratory cycle. Graphs are colour-coded for the delta- and theta-frequency bands (dark- and light-blue colours respectively), which are the EEG's primary frequency bands for which we observed statistically significant PAC (Figure 2C-D). Shaded regions indicate standard error over recordings. PAC was estimated for EEG channels FCz and Cz. Grey waves at the top and grey bar at the bottom indicate the phase of the respiratory cycle. Coloured dots indicate points that are statistically significant (alpha level was set to 0.001).* ***B)*** *Corresponding statistical significance analogous to the coloured dots at the top of panel A (black dashed line indicates the alpha level).* ***C)*** *Spatial map of the cross-frequency phase-slope index between respiratory phase ($\omega_{respiratory\ phase}$) and EEG amplitude ($\omega_{EEG\ amplitude}$) (see Figure S2 for the pan-spectral estimates) and* ***D)*** *its statistical significance (reported on a logarithmic scale). The spectra show the mean over all recordings.*

Standard coherence (i.e., our PAC measure) only quantifies linear coupling in a bidirectional manner and cannot directly show any directionality, meaning that it is not possible to distinguish whether cortical activity precedes or follows respiratory activity. To unravel whether the cortical activity drives respiration or *vice versa*, we employed a cross-frequency version of the phase-slope index[13]. This revealed that delta- and theta-band EEG amplitude leads respiratory activity (Figure 3C), demonstrating that the cortical activity drives respiration. We observed the strongest phase-slope index around central areas, in particular for channel FCz (Figure 3D). Moreover, in line with the PAC analysis (Figure 2), phase-slope index was not statistically significant for alpha- and beta-band frequencies (Figure S2).

Considering the cortico-respiratory coupling's spatial location, its timing in the respiratory cycle and its directionality of action leads us to conclude that this coupling observed represents the respiratory drive from higher-order motor centres, primarily encompassing the cortical motor areas. Structurally, the motor cortex connects to

respiratory muscles both directly and indirectly[18]. This allows for functional control over respiratory muscles (comprising diaphragm and intercostal muscles) via the corticospinal[19] and reticulospinal tracts[20]. In cat preparations, transection of the ventrolateral part of the spinal cord (which essentially blocks the brainstem input to respiratory motoneurons) does not prevent cortical stimulation from eliciting responses in the respiratory intercostal muscle motoneurons, signifying the cortico-spinal drive[20]. Such motor-evoked potentials can also be elicited by stimulating the cortical motor areas of humans using transcranial magnetic stimulation[21,22].

Whilst we believe that it is likely that our coupling reflects cortical motor drive, one might also argue that cortico-respiratory coupling could reflect changes in blood flow or oxygen saturation which induces 'coupling', where blood flow/oxygen saturation acts as a confounding factor essentially modulating respiratory and brain activity in a cycle-dependent manner. It is, however, unlikely that this is the case for our results given the focal spatial localisation around central electrodes and narrow spectral bandwidth comprising EEG frequencies between 1-8 Hz. Moreover, the amplitude modulation of blood flow reveals a different time-frequency representation over the respiratory cycle (Figure S3) when compared to cortical activity (Figure 2A).

To further our understanding of whether the coupling in infants relates directly to central motor drive, future work should examine potential coupling in the cortico-muscular domain. Electrical activity of the diaphragm (Edi) in infants can be recorded using an Edi catheter[23], as is used in ventilators with neurally adjusted ventilatory assist[24]. Correlating this electromyographic (EMG) activity from the diaphragm with brain activity (e.g., EEG) will enable cortico-muscular assessments that can quantify whether oscillatory components in the EEG and diaphragmatic EMG are phase-locked. It is of particular interest whether linear coherence can be observed in the frequency range of 1-8 Hz, which is expected from our PAC findings presented in Figure 2. Interestingly, such coherence analysis has been conducted at the muscular level in rodents. Invasively recording activity from the diaphragm and intercostal muscles revealed strong inter-muscular coherence in the lower frequencies between 1 and 8 Hz[25]. Future work should also establish to what extent the cortical motor areas provide control over other respiratory functionalities such as altering respiratory rate. In the current investigation, we focused on how cortical amplitude directly related to the respiratory phase but phase-amplitude coupling of cortical activity has also been

214 revealed to play a role in adjusting respiratory rate[26] (i.e., respiratory rate rather than
215 individual breaths).

216

217 *Cortico-respiratory coupling relates to apnoea in infants*

218 Having identified significant cortical-respiratory coupling in infants, we then tested our
219 main hypothesis - that the strength of the cortico-respiratory coupling is negatively
220 associated with apnoea. Apnoea of prematurity can lead to periods of physiological
221 instability when apnoea is accompanied by bradycardia and desaturation[5]. These
222 episodes may have long-term neurological effects[4] and, hence, it is important to better
223 understand the pathophysiology. In addition to pulmonary immaturity, apnoea of
224 prematurity has been primarily attributed to immaturity and weakened neural drive
225 from the respiratory centres in the brainstem. These respiratory centres are principally
226 located in the medulla oblongata and pons within the brainstem, which ensure the
227 periodical activation of lung muscles to enable respiration. However, as our results
228 indicate, further central input to respiration is provided by higher-order brain areas
229 such as the cortical motor areas.

230 To test our hypothesis, apnoea rate was estimated over a time window of up to
231 24 hours (infants have their vital signs continuously monitored at the neonatal care
232 unit), whereas cortical-respiratory coupling was assessed on the same day from EEG
233 recordings with an average duration of 1.7 ± 0.6 hours. Choosing different time
234 windows ensured that any relationship between apnoea rate and cortical-respiratory
235 coupling was not the mere result of the number of breaths included in the analysis.
236 For instance, when the same respiratory signal is used in both analyses, a higher
237 apnoea rate would mean that fewer windows are used for the PAC estimation which
238 could result in an increased magnitude. We selected PAC pooled over EEG channels
239 Cz and FCz since their coupling magnitudes were the highest.

240 We found support for our hypothesis that apnoea and cortico-respiratory-
241 coupling are related. PAC was negatively associated with apnoea rate ($t(98)$:-2.28,
242 p:0.02, ρ:-0.22; Figure 4A; this relationship was also identified when focusing on
243 individual channels [Figure S4] and preterm infants younger than 36 weeks [Figure
244 S5]). This suggests that a stronger cortico-respiratory coupling may reduce the
245 likelihood of experiencing apnoeas in infants. This negative association provides

further support that our measures of cortico-respiratory coupling (Figures 2-3) are related to central motor drive. In this case, cortical motor activity assists the brainstem's central pattern generators (CPGs) to establish increased control over the respiratory muscles, which in our analysis, is reflected as an association with apnoea rate. The motor cortex can exert coordination over the CPGs through its downstream projections to the thalamus[27], which, in turn is connected to the preBötzinger complex (the structure that primarily hosts the CPGs[28]).

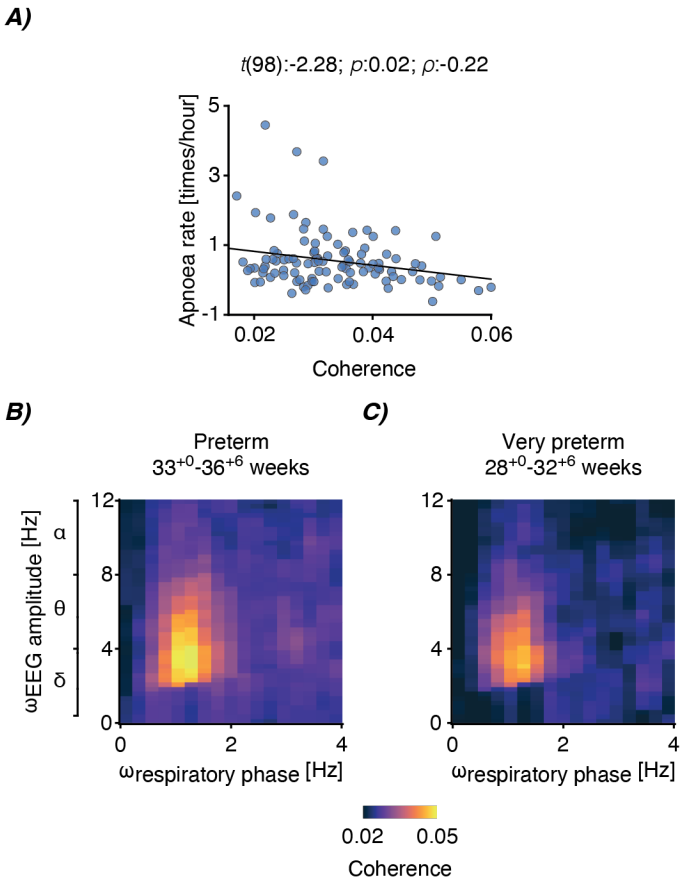

Figure 4. Cortico-respiratory coupling dependency on apnoea rate and postmenstrual age. A) *Relationship between apnoea rate and statistically significant phase-amplitude coupling (PAC; defined as coherence encompassing delta- and theta-band frequencies). Each blue dot is the data of an individual recording (data of 68 infants were included from 104 test occasions). Black linear graph is the best fit of the linear mixed-effects model (fixed factors: coupling averaged over statistically significant samples from channels FCz and Cz [see Figure S4 for channel-specific relationships], data length to determine apnoea rate, mode of ventilation, and postmenstrual age; random factor: infant). Panel title contains the test statistic of the predictor's regression slope (t), its significance (p), and partial correlation coefficient (ρ).* ***B-C)*** *PAC spectra between the respiratory phase ($\omega_{respiratory\ phase}$) and EEG amplitude ($\omega_{EEG\ amplitude}$) for* ***B)*** *preterm (58 recordings; 33.0-36.86 weeks), and* ***C)*** *very preterm (28 recordings; 28.0-32.86 weeks) infants. Both spectra are averaged over recordings and EEG channels FCz and Cz.*

In theory, the strength of cortical-respiratory coupling and its inverse relationship with apnoeas could reflect a shared relationship with PMA, where both

coupling strength and apnoea rate change depending on PMA. The apnoea rate is expected to decrease with increased PMA across a broad age range[29]. It is also plausible that the strength of cortico-respiratory coupling (i.e., PAC magnitude) may change with PMA, with younger (i.e., preterm) infants potentially having weaker coupling. However, in our dataset, we observe statistically significant PAC in preterm infants (33^{+0}-36^{+6} weeks^{+days}; Figure 4B), and very preterm infants (28^{+0}-32^{+6} weeks^{+days}; Figure 4C). Moreover, PAC and apnoea rate were not significantly associated with PMA (Figure S6A-B, linear mixed-effects analysis). Similarly, PAC and apnoea rate were not significantly related to postnatal age (PNA; defined as the age in weeks from birth; Figure S6C-D). Whilst future studies should investigate the relationship between cortico-respiratory coupling and age across an even broader age range, the lack of significant associations here suggests that the relationship we observed between apnoea rate and cortico-respiratory coupling is not determined by age.

It is known that most apnoeas in infants occur during active sleep[6,30] and delta- and theta-band frequencies in EEG are strongly related to sleep state[31]. We speculate that cortico-respiratory coupling may change depending on sleep state, giving rise to more episodes of apnoea during certain sleep stages. Indeed, adult studies have suggested that cortical respiratory drive may occur predominantly during wakefulness[6,32]. Future work should study the effects of sleep stages on cortico-respiratory coupling to shed further light on the role of cortical motor drive for respiration in infants.

In summary, we demonstrate the existence of cortico-respiratory coupling in newborn infants. We believe that this coupling is related to central motor drive due to its spatial location (predominantly over frontocentral EEG channels), timing (during inspiration) and directionality (with the cortex driving respiration). Consistent with our hypothesis, we found a significant relationship between apnoea rate and coupling strength, with infants with stronger cortico-respiratory coupling less likely to experience apnoea. This has far-reaching implications for our mechanistic understanding of apnoea of prematurity as it demonstrates that cortical motor areas play a pivotal role in apnoea in infants.

Data and code availability

- Due to ethical restrictions, the raw data that support the findings of this study are available on reasonable request. Requests can be made directly to the corresponding author (caroline.hartley@paediatrics.ox.ac.uk) or directed to the Paediatric Neuroimaging Group (University of Oxford) through the institutional email: neonatal.research@ouh.nhs.uk. Researchers will be required to agree to destroy the data within the time limits stipulated in the ethically approved study protocols. Upon agreement, data will be provided within 1 month.
- Summary-level data (before regression model adjustments of Figure 4), and channel- and recording-specific PAC spectra are provided at https://gitlab.com/paediatric_neuroimaging/cortico_respiratory_coupling.
- The codes for all analyses are available at: https://gitlab.com/paediatric_neuroimaging/cortico_respiratory_coupling. The algorithm for inter-breath interval detection is online at: https://gitlab.com/paediatric_neuroimaging/identify_ibi_from_ip.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

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Author contributions

Conceptualization: CSZ and CH; **Data curation:** FU, SR, OF, and CH; **Formal analysis:** CSZ; **Funding acquisition:** CH; **Investigation:** FU, SR, and OF; **Methodology:** CSZ; **Project administration:** CH; **Software:** CSZ; **Resources:** EA,

KTSP and SFF; **Supervision:** CH and EA; **Visualization:** CSZ; **Writing – original draft:** CSZ; **Writing – review & editing:** all authors

Declaration of interests

The authors declare no competing interests.

337 ***Material & Methods***

338

339 *Participants and study design*

340 This study was conducted at the Newborn Care Unit of the John Radcliffe Hospital,
341 Oxford University Hospitals NHS Foundation Trust, Oxford, UK. The National
342 Research Ethics Service approved the study (references: 19/LO/1085; 12/SC/0447).
343 Eligible families were approached and given verbal and written details about the study.
344 Parents provided written consent to take part in the study. The study conformed to the
345 standards set by the Declaration of Helsinki and Good Clinical Practice.

346 Clinically stable infants aged between 28- and 42-weeks postmenstrual age
347 (PMA) at the time of the test occasion were eligible to be included in the study. Infants
348 were excluded if they had intraventricular haemorrhage grades III or IV, hypoxic
349 ischaemic encephalopathy, congenital malformations, there was a known history of
350 maternal substance misuse during pregnancy, or at the time of the study, they were
351 receiving mechanical ventilation, opioid analgesics, or antibiotics (as a surrogate
352 marker of infection). These conditions are known to alter respiratory and brain activity.
353 We enrolled 74 infants who were recorded on 123 separate occasions (range: 1-8
354 recordings per infant). Fifty-one infants were included in one or two test occasions; 23
355 infants took part in the ongoing *Breathing and Brain Development* study where they
356 are studied approximately once a week whilst in the Newborn Care Unit (included on
357 3±2 [median ± interquartile range] occasions). Demographic details of all infants can
358 be found in Table 1. Parts of this dataset have been reported earlier in Zandvoort et
359 al. [33] to address a different research question.

360

Factors	
Age	
Postmenstrual age at recording (weeks)	34.5 ± 2.6
Gestational age at birth (weeks)	31.1 ± 3.4
Postnatal age at recording (weeks)	3.4 ± 2.2
Birthweight (g)	1,726 ± 816

Sex	
Females	27
Males	47
Mode of delivery	
Normal vaginal delivery	16
Vaginal assisted (ventouse/forceps/kiwi)	4
Elective Caesarean section	7
Emergency Caesarean section	47
Apgar scores	
Apgar at 1 minute	7.2 ± 2.2
Apgar at 5 minutes	9.1 ± 1.4
Apgar at 10 minutes	9.7 ± 0.8
Respiratory distress syndrome	
Ongoing condition	39
Past treated	48
No	36
Persistent pulmonary hypertension	
Past treated	5
No	118
Infection	
None	48
Treated	75
Respiratory support	
High flow therapy	25
Low flow therapy	16
Spontaneous ventilation	82
Caffeine received on the day of the EEG recording	
Yes	59
No	64

Table 1. Infant demographics. *Reported values are mean and standard deviation or number of babies or recordings. Values are reported over recordings for ages (postmenstrual, gestational, and postnatal), respiratory distress syndrome, persistent pulmonary hypertension, infection and respiratory support. Birthweight, sex, mode of delivery, and Apgar scores are provided over infants.*

366 *Data recordings*

367 During each test occasion, approximately one to two hours of simultaneous
368 electroencephalography (EEG) and vital signs (respiration, heart rate and oxygen
369 saturation) were recorded. We continuously recorded the vital signs throughout their
370 hospital stay of the infants who took part in the *Breathing and Brain Development*
371 study.

372 Vital signs were recorded for each infant using Phillips IntelliVue MX800 or
373 MX750 monitors. These data were continuously downloaded from the vital signs
374 monitor using an electronic data capture software (iXtrend, ixitos, Germany). Both
375 electrocardiograph (ECG; sampling frequency: 250 Hz) and impedance
376 pneumography (IP; sampling frequency: 62.5 Hz) were recorded with three electrodes
377 placed on the infant's chest. Peripheral photoplethysmography was recorded at 125
378 Hz from a probe placed on the infant's foot or hand.

379 EEG was recorded from DC to 400 Hz using a SynAmps RT 64-channel
380 headbox and amplifiers and CURRYscan7 neuroimaging suite (Compumedics
381 Neuroscan) at a sampling rate of 2 kHz. Eight electrodes were placed at Cz, CPz, C3,
382 C4, Oz, FCz, T3, and T4. The ground electrode was placed at FPz and the reference
383 electrode at Fz. The infant's scalp was cleaned with NuPrep gel (D.O. Weaver and
384 Co., Aurora, USA) to achieve impedances of 5-10 kΩ. A conductive paste (Elefix EEG
385 paste, Nihon Kohden, Tokyo, Japan) was used to attach disposable Ag/AgCl cup EEG
386 electrodes (Ambu Neuroline, Ballerup, Denmark) to the infant's scalp. EEG was
387 recorded for an average of 1.7 ± 0.7 hours (mean ± SD). ECG was co-registered with
388 our EEG recordings using a single electrode (Ambu Neuroline, Ballerup, Denmark)
389 placed on the infant's chest (referenced to Fz).

390 To synchronise the vital signs and EEG recordings, we registered
391 approximately 30 annotations simultaneously on both recordings using a bespoke
392 triggering device (the PiNe box)[34]. Annotations were used to mark resting state as well
393 as stimulus-evoked activity (these responses were used in other studies to investigate
394 infant brain maturity – see Zandvoort *et al.* [33] – but were used here only to synchronise
395 the EEG and vital signs recordings). The PiNe box enables synchronisation with an
396 average precision of 105 ± 14 ms (mean ± SD)[34]. Annotation timings were matched
397 between both vital signs and EEG recordings. Next, to further improve synchronisation,

398 we utilised the ECG signals which were recorded on both the vital signs and EEG
399 recording devices. To do so, ECG epochs were created from -10 to 10 seconds around
400 each annotation. We aligned the ECG signals around each of these annotation epochs
401 using cross correlations, providing a temporal delay between vital signs and EEG[34].
402 Temporal synchronisation between vital signs and EEG recordings was achieved by
403 taking the median of the time differences across all annotations.

404 From the total of 123 recordings, we excluded 19 recordings due to (a) missing
405 IP recordings (5 recordings), (b) missing annotations (9 recordings), and (c)
406 inaccuracies in EEG-IP syncing (5 recordings; standard deviations over annotations
407 exceeding 1 sec for annotation alignment or 0.1 sec during ECG alignment). Hence,
408 data comprising 104 recordings from 68 infants were used in the analysis.

409

410 *Breath and apnoea identification*

411 IP signals were processed to identify breaths (including apnoeas). To this end, we
412 used an algorithm validated to detect inter-breath intervals in infants[14]. Briefly, the
413 algorithm attenuates noise introduced by movements and cardiac activity by filtering
414 the signal. An adaptive amplitude threshold then identifies individual breaths. This
415 threshold was set at 0.4 times the standard deviation of the signal over the preceding
416 15 breaths. Finally, a support vector machine algorithm was applied to all long (here,
417 15 seconds or greater) inter-breath intervals to separate episodes of apnoea
418 (cessation of respiration) and periods of low amplitude erroneously identified as
419 apnoeas due to noise or shallow breathing. The inter-breath intervals marked as
420 noise/shallow breathing were discarded from further analysis. The model was trained
421 and validated on IP signals acquired during a clinical trial[35], which was a completely
422 independent data sample to the one used in the current study.

423

424 *Phase-amplitude coupling*

425 In line with Osipova *et al.* [12], we defined phase-amplitude coupling (PAC) as cross-
426 frequency coherence between a low-frequency phase and high-frequency amplitude.
427 Note that the term “phase-amplitude coupling” is used interchangeably in the literature
428 to mean the coupling principle of the statistical relationship between phases and

429 amplitudes as well as the actual measure to detect this. We reserve the abbreviation
430 for the latter. For our PAC measure, this meant that coherence was computed between
431 the filtered IP signal and instantaneous amplitude of the EEG signal at some higher
432 frequency (i.e., the second-order moment between the IP phase and EEG amplitude).
433 More formally, in our case PAC was defined as:

434

$$C(f_1, f_2) = \frac{|\sum_{n=1}^{N} X(f_1) * Y(f_1, f_2)^*|}{\sqrt{\sum_{n=1}^{N} |X(f_1)|^2 * \sum_{n=1}^{N} |Y(f_1, f_2)|^2}} \qquad \text{Eq. 1}$$

435

436 with X being the IP signal at frequency f_1, Y the instantaneous EEG amplitude, and *
437 the complex conjugate. This instantaneous amplitude was obtained using the
438 magnitude of the Hilbert transform after narrowly filtering the data at frequency f_2. This
439 component is often filtered with a bandwidth that relates to the low-frequency
440 component f_1. Following earlier recommendations, the filter bandwidth of the high-
441 frequency component (at f_2) was equal to a single time the frequency of the low-
442 frequency component f_1[36] (e.g., when f_1 equals 5 Hz the filter bandwidth of f_2 is set to
443 5 Hz).

444 Data were segmented into 4-sec windows (50% overlap), which meant that the
445 frequency resolution was equal to 0.25 Hz (allowing to capture low delta-band
446 frequencies), which comprised around four breaths. To focus on regular respiration,
447 we discarded epochs which contained fewer than three detected breaths. An epoch
448 was also removed from further analysis when its standard deviation exceeded 3 times
449 the mean or median of the standard deviation of all epochs (which was done
450 separately for the EEG and IP). Finally, we rejected any epoch containing an absolute
451 value greater than 10 times the mean or median of the standard deviation of all epochs.
452 Before cross-spectrum computation, EEG and IP epochs were convolved with a
453 wavelet constructed from a Hanning window.

454

455 *Changes in PAC throughout the respiratory cycle*

456 To investigate how cortico-respiratory coupling changes throughout the respiratory
457 cycle, we estimated PAC at 50 equally spaced timepoints within the respiratory cycle.

To this end, we time-locked both EEG and IP to each inter-breath interval as identified by the algorithm of Adjei *et al.* [14]. At each respiratory cycle phase, cross- and auto-spectra were obtained to estimate PAC over all breaths within a recording. We limited the analysis to EEG channels FCz and Cz, because they exhibited the highest PAC magnitude (Figure 2), as well as statistically significant frequency-frequency samples relative to the surrogate PAC (see Figure 2D for the *p*-values of this statistical comparison). PAC was computed within the different frequency bands (delta: 0.5-4 Hz; and theta: 4-8 Hz) to see whether its modulation was specific for each frequency band. The same preprocessing steps apply as for PAC computation described earlier apart from the fact that data were segmented into 2/3 sec windows (so that temporal changes would appear within the respiratory cycle).

Directionality of phase-amplitude coupling

To assess whether the cortical activity was preceding or trailing relative to the respiratory activity, we employed a cross-frequency version of the phase-slope index. Briefly, this metric estimates the phase lags over narrow frequency ranges and quantifies whether they increase or decrease over this range by estimating the phase-frequency slopes. Increasing or decreasing slopes signify changing time lags depending on the frequency of the oscillation and can therefore be indicative of whether a signal leads or trails another signal. We used the phase-slope index (Ψ) as proposed by Jiang *et al.* [13], which was defined as:

$$\Psi(f_1, f_2) = imag\left(\sum_{f_2-\frac{\beta}{2}}^{f_2+\frac{\beta}{2}} \tilde{C}^*(f_1, f_2)\, \tilde{C}(f_1, f_2 + \Delta f)\right) \qquad \text{Eq. 2}$$

with $\tilde{C}$ being the coherency (i.e., the normalised cross-spectrum) of C defined in Eq. 1. Δf is the frequency resolution and β the bandwidth used to estimate the phase slope. In line with previous recommendations by Jiang *et al.* [13], we set β to 1 Hz (i.e., four times the frequency resolution).

Surrogate analysis

To assess the statistical significance of PAC (i.e., coherence) and phase-slope index at each of the EEG channels, we performed a surrogate analysis under the null hypothesis that the means of the true and surrogate PAC (and phase-slope index) were equal. The surrogate analysis consisted of randomly shuffling epochs of the EEG amplitude after which PAC was computed with the IP signal. The random shuffling of the EEG amplitude epochs destroys any temporal relationships whilst retaining the signals' characteristics. We obtained a surrogate spectrum for each recording. Next, the true and surrogate spectra were statistically compared on a sample-by-sample basis using dependent *t*-tests[37] (using the FieldTrip toolbox – version 20210325). This comparison was performed over all recordings. Statistical significance was defined using the false discovery rate correction (with an alpha level set at 0.001).

Statistical analysis of relationships between cortico-respiratory coupling and apnoea

We tested our main hypothesis that PAC is negatively associated with apnoea rate. Vital signs were continuously monitored for the infants that took part in the *Breathing and Brain Development* study, which allowed us to analyse their vital signs in longer time windows than the EEG. We here selected up to 24 hours of vital signs from the day of the EEG recording (mean ± standard deviation: 9.5 ± 9.9 hours) and expressed this as a rate per hour. We then constructed linear mixed-effects models to study the relationships between PAC and apnoea rate. We included the coherence magnitude as a fixed factor, which was averaged over the statistically significant samples from channels FCz and Cz (see Figure S4 for the channel-specific relationships). As fixed effects, we included (1) data length of the respiratory signal, (2) mode of ventilation ('Self-ventilating', 'Low flow', 'High flow'; Figure S7), and (3) PMA (Figure S6). Infant ID was included as a random effect. Data length, mode of ventilation and PMA were included as confounding factors. P-values (for the regression coefficient of PAC) and partial correlation coefficients were reported from these models. The normality assumption was verified with Q-Q plots. We also tested the linearity assumption (see Supplementary text). For visualisation (Figure 4), we used the plotAdjustedResponse function in MATLAB to adjust for the fixed factors (calculating a separate model without

517 the random effect of an infant). Regression lines are visualised by calculating the line-
518 of-best-fit on the adjusted data.

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