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BASIC SCIENCE ARTICLE

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The effect of vibrotactile stimulation on hypoxia-induced irregular breathing and apnea in preterm rabbits

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BACKGROUND: Manual tactile stimulation is used to counteract apnea in preterm infants, but it is unknown when this intervention should be applied. We compared an anticipatory to a reactive approach using vibrotactile stimulation to prevent hypoxia induced apneas.

METHODS: Preterm rabbit kittens were prematurely delivered and randomized to either group. All kittens breathed spontaneously with a positive airway pressure of 8 cmH_2O while they were imaged using phase contrast X-ray. Irregular breathing (IB) was induced using gradual hypoxia. The anticipatory group received stimulation at the onset of IB and the reactive group if IB transitioned into apnea. Breathing rate (BR), heart rate (HR) and functional residual capacity (FRC) were compared.

RESULTS: Anticipatory stimulation significantly reduced apnea incidence and maximum inter-breath intervals and increased BR following IB, compared to reactive stimulation. Recovery in BR but not HR was more likely with anticipatory stimulation, although both BR and HR were significantly higher at 120 s after stimulation onset. FRC values and variability were not different.

CONCLUSIONS: Anticipated vibrotactile stimulation is more effective in preventing apnea and enhancing breathing when compared to reactive stimulation in preterm rabbits. Stimulation timing is likely to be a key factor in reducing the incidence and duration of apnea.

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IMPACT:

- Anticipated vibrotactile stimulation can prevent apnea and stimulate breathing effort in preterm rabbits.
- Anticipated vibrotactile stimulation increases the likelihood of breathing rate recovery following hypoxia induced irregular breathing, when compared to reactive stimulation.
- Automated stimulation in combination with predictive algorithms may improve the treatment of apnea in preterm infants.

INTRODUCTION

Spontaneous breathing in preterm infants typically follows a periodic and irregular pattern, reflecting the immaturity of their lungs, central respiratory control centers and muscles. Breathing pauses that last more than 10 to 20 seconds are referred to as apnea and are observed in most preterm infants born at <30 weeks gestation.[1] Apnea can develop into a significant clinical problem as these episodes are often accompanied by bradycardia and/or hypoxia, which consequently increases the risk of lung, eye and brain injury.[2,3]

Caffeine treatment and non-invasive respiratory support are commonly used to reduce the occurrence of apneic episodes,[4] but do not fully prevent it. In order to restore breathing, caregivers must promptly intervene by providing an escalating sequence of interventions including tactile stimulation, additional supplemental oxygen, positive pressure ventilation and, in more severe cases, intubation.[5] Manually applied tactile stimulation is arguably the most frequent and important intervention used in response to apnea and has been recommended and applied in clinical practice for many years. However, manual interventions come with response delays,[6] which makes treatment of apnea on demand an ongoing challenge.

Mechanical and automated tactile stimulation can avoid the delay in response and so has the potential to avert or shorten the duration of apnea, prevent the onset or exacerbation of bradycardia and hypoxia, and hence mitigate the need to escalate the required intervention. These benefits could be further enhanced when stimulation is provided when apnea is anticipated. Although mechanical, vibrotactile stimulation has been studied in several small clinical trials, showing positive results

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when applied in both a continuous and reactive manner,[7–11] the benefits of an anticipatory response have never been assessed.[12]

The aim of this study was to investigate whether an anticipatory stimulation approach is more effective at stimulating breathing and preventing apnea than a reactive stimulation approach in preterm rabbit kittens. Therefore, we compared the effect of mechanical vibrotactile stimulation in response to hypoxia-induced irregular breathing (IB) with stimulation in response to apnea. We hypothesized that the anticipatory approach would be more effective than the reactive approach.

METHODS

All animal procedures were approved by the SPring-8 Animal Care and Monash University's Animal Ethics Committees. The study was conducted in experimental hutch 3 of beamline 20B2 in the Biomedical Imaging Centre at the SPring-8 synchrotron in Japan.

Experimental procedure

Eight pregnant New Zealand White rabbits were initially sedated using propofol (8 mg/kg iv bolus, Rapinovet, Merck Animal Health) at 29 days gestational age (GA; term ≈32 d) to administer a spinal block using 2% lignocaine (4 mg/kg) and 0.5% bupivacaine (1 mg/kg), as previously described.[13] Following the induction of spinal anesthesia, sedation was maintained in the pregnant doe with intravenous infusion of midazolam (1 mg/kg/h) and butorphanol (0.5 mg/kg/h). The rabbit's heart rate (HR), SpO_2, breathing rate (BR) and hind quarter reflexes were monitored during delivery of the rabbit kittens.

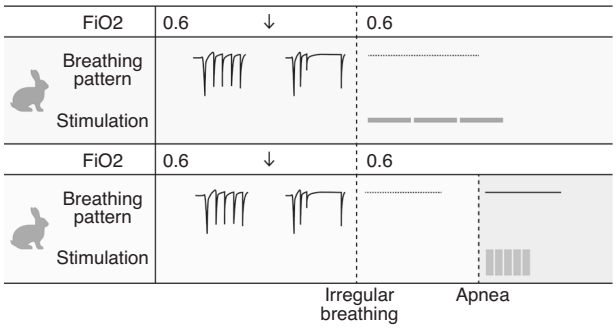

Fig. 1 Experimental procedure for the anticipatory and reactive group. In the anticipatory group (green) the FiO_2 was increased to 0.6 and stimulation was applied in response to irregular breathing. In the reactive group (orange) FiO_2 was increased in response to irregular breathing but stimulation was only applied if irregular breathing transitioned into apnea.

Rabbit kittens were randomized to either the anticipatory or reactive approach group prior to delivery by caesarean section. After exteriorization of each kitten from the uterus, an esophageal tube was inserted to measure intrathoracic pressure and a custom-made mask was fitted over the head of each kitten to administer continuous positive airway pressure (CPAP) and oxygen. Naloxone (1 mg/kg) and anexate (10 µg/kg) were administered intraperitoneally to each kitten to reverse the effects of maternally administered butorphanol and midazolam, and caffeine citrate (20 mg/kg) was given to stimulate breathing. After cutting the umbilical cord, the kittens were transferred into the imaging hutch and placed lateral on a custom-made, heated, stimulation device. Electrocardiogram (ECG) leads were attached and the facemask was connected to a purpose-built ventilator.[14] to administer an initial CPAP of 15cmH_20 and a fraction of inspired oxygen (FiO_2) of 0.6. As the lungs aerated and the breathing pattern stabilized the CPAP level was decreased to 8 cmH_20 and remained at this level for the rest of the experiment. Phase contrast (PC) X-ray imaging of the kittens then commenced for measuring lung air volumes. Subsequently FiO_2 was reduced in a stepwise manner, with steps off ±0.1, to reduce oxygenation levels and hence induce an unstable irregular breathing (IB) pattern. Once IB was observed, characterized by a variable breathing pattern and amplitude, the FiO_2 was returned to 0.6 in both groups. At this time, the kittens in the anticipatory approach group received vibrotactile stimulation. Kittens in the reactive approach group only received stimulation if and at the moment that IB transitioned into apnea (Fig. 1).

After delivery of the final kitten or at the conclusion of the imaging period, all does and kittens were euthanized with an overdose of sodium pentobarbitone (> 100 mg/kg) administered intravenously (doe) or intraperitoneally (kittens).

Vibrotactile stimulation

A custom-made mechanical stimulation device (Fig. 2) was used to remotely administer vibrotactile stimulation while the kittens were being imaged. This device consisted of a metal box with an enclosed speaker and a small rod that connected the conus of the speaker, through a hole in the box, to a point in contact with the kitten. The kitten was placed laterally on the box, with the contact point located at the level of the thorax. The speaker was connected to a laptop via an amplifier and was actuated by playing an audio file, allowing control of the signal frequency and displacement (amplitude) of the contact point. Using an optical sensor placed inside the metal box, the frequency and amplitude of the stimulations were recorded in Labchart (Powerlab, ADInstruments; Sydney, Australia).

The stimulation in the anticipatory approach group consisted of three 30 seconds blocks of vibration, generated by a sinusoidal tone with a frequency of 100 Hz and an amplitude of 0.2 mm. Although it was our initial intent to solely compare the effect of timing, we decided to adjust the length and amplitude of the reactive stimulation in order to increase the likelihood of recovery and to more closely match the characteristics of the stronger manual reactive stimulation that is usually required to restore breathing in apneic kittens. Vibrotactile stimulation in the reactive approach group consisted of multiple vibrations that were 5 seconds in duration with a frequency of 100 Hz and an amplitude of 1.1 mm.

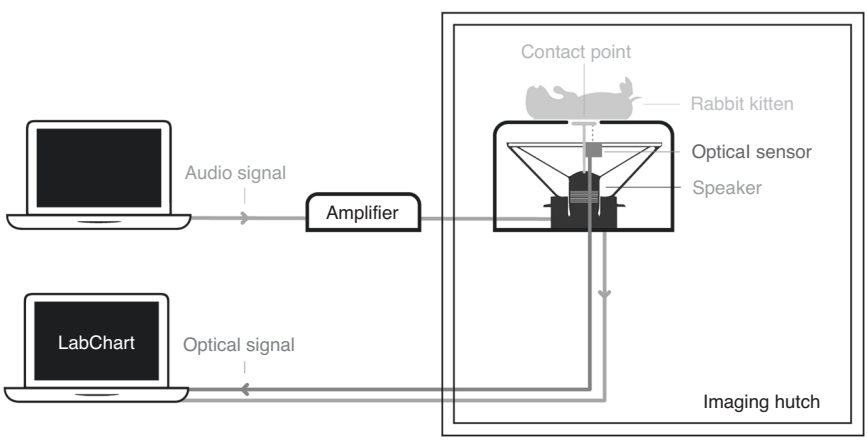

Fig. 2 Set-up of the vibrotactile stimulation device. The device consists of an amplifier and a metal box with a speaker and optical sensor inside. A rod is connected to the conus of the speaker which transmits the vibration of the conus to the skin of the kitten.

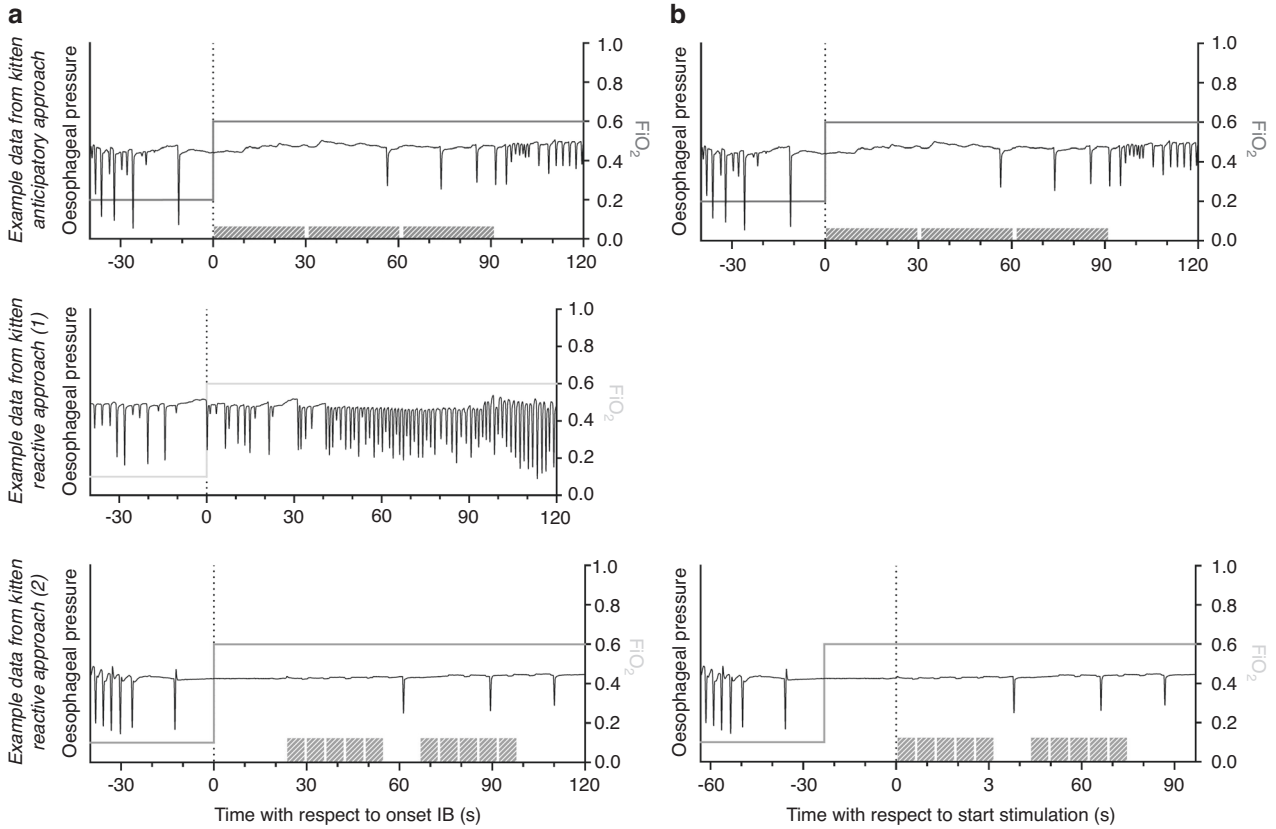

Fig. 3 Visualization of data analysis. The upper tracings are from a kitten in the anticipatory group and the middle and lower tracing are from kittens in the reactive group. Analysis (**a**): comparing anticipatory approach versus reactive approach by using the onset of IB ($t = 0$) as reference point. Both groups receive 0.6 FiO_2 at the onset of IB, but only the kitten from the anticipatory group (upper tracing) receives stimulation simultaneously. Kittens from the reactive group (middle and lower tracing) only receive stimulation if IB transitions to apnea (only lower tracing). Analysis (**b**): comparing anticipatory approach versus reactive approach by using the start of stimulation ($t = 0$) as reference point. For the kittens in the anticipatory group this timepoint is the exact same point as the onset of IB from analysis (**a**), but for the kittens of the reactive group the reference point is shifted. This analysis only includes kittens from the reactive group that received rescue stimulation once IB transitioned in apnea (only the lower tracing).

PC X-ray imaging

High resolution PC X-ray imaging was used to measure lung gas volumes using a power spectral analysis, as described previously by Leong et al.[15] The study was conducted over two beamtimes due to the number of animals used. Monochromatic synchrotron X-ray radiation was used to illuminate the lungs at an energy of 24 keV. A lens-coupled Hamamatsu ORCA Flash detector (C11440-22C; effective pixel size 15.3 micron) coupled to a 25 micron thick Gadox phosphor (P43) recorded the phase contrast images with an exposure time of 20 ms and a frame rate of 10 Hz. The source-to-kitten distance was ~210 m, and the kitten-to-detector distance was 2 m.

Data analysis

Inter-breath intervals (IBI) and R-R intervals (RRI) were obtained from the recorded intrathoracic pressures and ECG signals in Labchart (Powerlab, ADInstruments; Sydney, Australia). BR were computed at 5 second intervals using a moving average over the previous 15 second period whereas HR were averaged over the previous 20 seconds also at 5 seconds intervals. Lung functional residual capacity (FRC) volumes were derived from the PC X-ray imaging and also averaged over a window of 25 seconds every 5 seconds. Motion artefacts due to the vibration of the kitten during stimulation blurred our the phase contrast required to measure lung volumes. This resulted in missing FRC averages for some time windows.

To compare the effect of an anticipatory versus a reactive stimulation approach, two comparisons between groups were performed using either the onset of IB or the onset of stimulation as a reference point (Fig. 3). The average overall BR, HR and FRC, and the coefficient of variation (COV) of these parameters were used to compare cardiorespiratory stability between groups with respect to the onset of IB. These metrics were computed using the raw values (IBI, RRI and FRC), starting 40 seconds before the onset of IB to 120 seconds thereafter. Cardiorespiratory responses to both stimulation approaches were assessed by, (i) the occurrence of apnea – defined as one breathing pause of ≥ 20 s or multiple consecutive pauses of >15 s, (ii) the maximum IBI and (iii) the area under the curve (AUC) for BR, HR and FRC, in the 120 seconds following the onset of IB. For the second comparison recovery percentages of BR and HR as well as the BR, HR, and FRC values at 120 seconds after the onset of stimulation were used. Successful recovery was defined as a BR or HR exceeding its level at IB onset within 120 seconds after stimulation onset. The two kittens (of 11) in the reactive group that did not become apneic were excluded from this analysis as they did not require or receive reactive stimulation.

Statistical Analysis

SPSS software version 23.0 (SPSS, Chicago, Illinois) was used to perform the statistical analyses.

Categorical data is presented as *n* (%) and compared between groups using Fishers exact test. Continuous data is presented as mean ± standard deviation (SD) if normally distributed or median and interquartile ranges (IQR) otherwise. Differences between groups were carried out on the (transformed) data accordingly, using the unpaired Student's t-test or Mann-Whitney U test. Tests were performed two-sided and p values < 0.05 were considered statistically significant.

RESULTS

Physiological data and corresponding PC X-ray images were collected from a total of 21 preterm rabbit kittens, delivered from eight does. Ten kittens were randomized in the anticipatory group and 11 in the reactive group. There were no significant differences between the two groups with regard to birth weight, administered

Table 1. Demographics.

	Anticipatory approach ($N = 10$)	Reactive approach ($N = 11$)	*P* value
Birth weight (grams)[a]	32.3 ± 5.6	32.5 ± 5.4	0.919
Caffeine dose (mL/kg)[a]	22.6 ± 4.1	20.6 ± 1.9	0.147
Timing of onset IB after birth (mm:ss)[a]	11:59 ± 04:06	13:22 ± 03:24	0.407
FiO_2 at IB onset (%)[b]	0.20 (0.10–0.37)	0.10 (0.05–0.20)	0.107

IB Irregular breathing, *FiO_2* fraction of inspired oxygen.
Data is presented as mean ± SD for normally distributed data (a) or median (IQR) for data that were not normally distributed (b).

Table 2. Comparisons with respect to onset of IB.

	Anticipatory approach $N = 10$	Reactive approach $N = 11$	*P* value
Overall BR (breaths/min)[b]	15.3 (10.4–19.5)	8.5 (7.1–19.4)	0.133
Overall IBI variability (%)[b]	60.5 (37.0–125.0)	108.0 (81.0–161.0)	0.149
AUC of BR after IB onset[b]	314.7 (123.6–424.3)	86.3 (46.6–142.2)	0.025
Incidence of apnea after IB onset(n)[c]	3/10 (30)	9/11 (82)	0.030
Maximum IBI after IB onset (sec)[b]	7.7 (5.1–30.8)	38.4 (15.5–73.9)	0.014
Overall HR (bpm)[a]	125.5 ± 50.2	93.0 ± 27.4	0.073
Overall RRI variability (%)[c]	18.7 ± 8.8	32.8 ± 15.8	0.038
AUC of HR after IB onset[a]	2892.0 ± 1248.8	2060.1 ± 640.2	0.066
Overall FRC (ml/kg)[b]	27.1 (22.3–30.0)	20.0 (17.1–25.5)	0.226
Overall FRC variability(%)[b]	1.8 (0.7–3.5)	1.9 (1.4–4.0)	0.536
AUC of FRC after IB onset[b]	657.4 (531.3–726.6)	527.6 (457.5–686.9)	0.902

BR breathing rate, *IBI* inter breath interval, *AUC* area under the curve, *IB* irregular breathing, *HR* heart rate, *RRI* R-R intervals, *FRC* functional residual capacity
Data is presented as mean ± SE (a), median (IQR) (b) or n (%) (c). *P*-values are based on transformed data.

dose of caffeine, timing of the onset of IB, and FiO_2 levels at the moment IB was observed (Table 1).

Comparisons with respect to onset of irregular breathing

Although BR and variability in IBI's over the entire analyzed period were not statistically different, kittens randomized to anticipatory stimulation tended to have higher BRs (15.3 (10.4–19.5) vs 8.5 (7.1–19.4) breaths/min, $p = 0.133$) and lower IBI variability (60.5 (37.0–125.0) vs 108.0 (81.0–161.0)%, $p = 0.149$) compared to the reactive stimulation group) (Table 2, Fig. 4a). After the onset of IB, 3/10 kittens in the anticipatory stimulation group became apneic as compared to 9/11 in the control group ($p = 0.030$). In addition, the maximum IBI was shorter (7.7 (5.1–30.8) vs 38.4 (15.5–73.9) seconds, $p = 0.014$), and the AUC for BR higher (314.7 (123.6–424.3) vs 86.3 (46.6–142.2), $p = 0.025$) in the anticipatory compared to the reactive stimulation group.

Overall HR and the AUC for heart rate following IB were higher in the anticipatory stimulation group, but these differences did not reach statistical significance (125.5 ± 50.2 vs 93.0 ± 27.4 bpm, $p = 0.073$, 2892.0 ± 1248.8 vs 2060.1 ± 640.2, $p = 0.066$) (Table 2, Fig. 4a). The variability in R-R intervals was significantly lower in the group that received anticipated stimulation (18.7 ± 8.8 vs 32.8 ± 15.8, $p = 0.038$).

There was no difference between overall FRC values (27.1 (22.3–30.0) vs 20.0 (17.1–25.5) mL/kg, $p = 0.226$), variability in FRC values (1.8 (0.7–3.5) vs 1.9 (1.4–4.0) mL/kg, $p = 0.536$) as well as AUC for FRC values following IB (657.4 (531.3–726.6) vs 527.6 (457.5–686.9) mL/kg, $p = 0.902$) between groups (Table 2, Fig. 4a).

Comparisons with respect to start stimulation

All kittens in the anticipatory group received stimulation when IB occurred, while only 9/11 kittens in the reactive stimulation group became apneic and so received stimulation. In the anticipatory stimulation group, BR recovered in 7/10 kittens compared to 1/9 kittens in the reactive stimulation group ($p = 0.015$) (Table 3). Similarly, HR recovered in 5/10 kittens in the anticipatory stimulation group versus 1/9 kittens in the reactive stimulation group ($p = 0.091$). Two minutes after the onset of stimulation, both BR and HR were still significantly higher in the anticipatory stimulation group (17.3 ± 13.7 vs 2.9 ± 1.8 breaths/min, $p = 0.009$, 123.9 ± 55.8 vs 64.7 ± 15.5 bpm, $p = 0.009$) (Table 3, Fig. 4b). FRC values were not different between groups (anticipatory: 27.7 (22.1–29.7) vs reactive: 19.1 (16.8–28.0) mL/kg, $p = 0.315$), although tended to be lower in the reactive group during the entire study.

DISCUSSION

In this study we have shown that vibrotactile stimulation can significantly reduce the incidence and duration of apnea and increase breathing rate if it is applied when breathing becomes irregular instead of waiting until after apnea onset. Together with the statistically insignificant but greater cardiorespiratory stability these data suggest that stimulating in anticipation of an impending apnea is considerably better than waiting for apnea to occur. With respect to the start of stimulation, anticipated stimulation led to recovery of breathing rate more often and resulted in a significantly higher breathing rate two minutes after stimulation onset when compared to reactive stimulation. Similarly, the heart rate two minutes following the start of stimulation was significantly higher when anticipatory stimulation was given. Interestingly, the FRC values were similar in both groups with little variability over the analyzed periods, despite the changes and differences in breathing rate between groups.

Our results support the generally accepted view that tactile stimulation can promote breathing and counteract apnea in preterm infants. Previous experimental studies have shown that stimulation of somatosensory receptors can trigger spontaneous

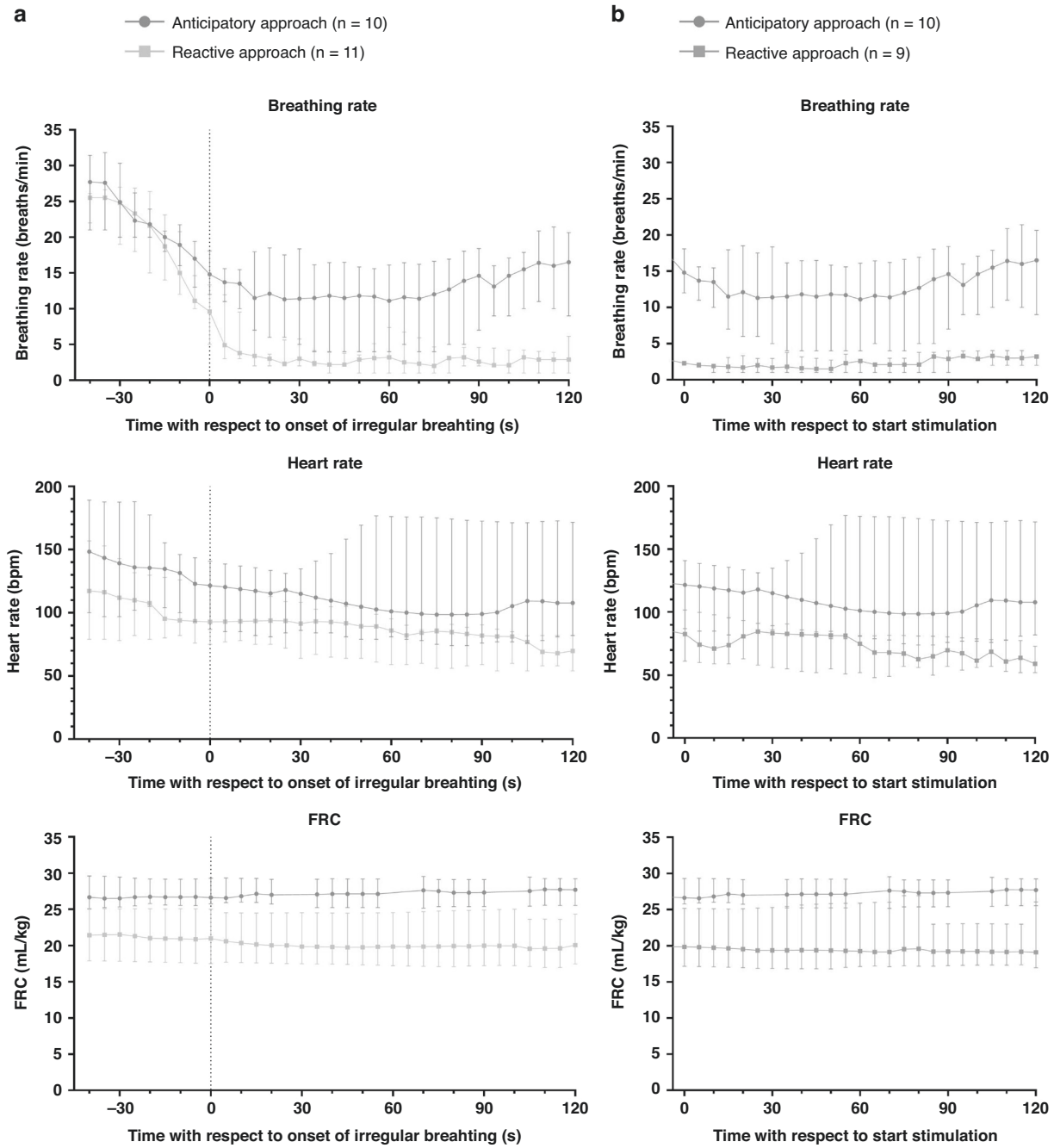

Fig. 4 Visualization of study results. Breathing rate, heart rate and FRC values over time for (**a**) anticipatory approach versus reactive approach, using the start of IB as reference point ($t = 0$) and (**b**) anticipatory approach versus reactive approach, using the start of stimulation as reference point ($t = 0$).

Table 3. Comparisons with respect to start stimulation.

	Anticipatory approach $N = 10$	Reactive approach $N = 9$	P-value
BR 120 s after stimulation onset (breaths/min)[a]	17.3 ± 13.7	2.9 ± 1.8	0.009
Incidence of BR recovery within 120 s after stimulation onset	7/10 (70)	1/9 (11)	0.015
HR 120 s after stimulation onset (bpm)[a]	123.9 ± 55.8	64.7 ± 15.5	0.009
Incidence of HR recovery within 120 s after stimulation onset	5/10 (50)	1/9 (11)	0.091
FRC 120 s after stimulation onset (ml/kg)[b]	27.7 (22.1–29.7)	19.1 (16.8–28.0)	0.315

BR breathing rate, *HR* heart rate, *FRC* functional residual capacity.
Data is presented as mean ± SE (a), median (IQR).

fetal breathing,[16,17] enhance respiratory drive after birth[18] and shorten the duration of induced apnea.[19] Our study also confirmed earlier reports that not all forms and methods of mechanical stimulation are (equally) effective.[19–21] In addition to stimulation characteristics such as type, intensity and location, we have now demonstrated that the effectiveness of stimulation is also influenced by timing. Although both groups were treated in exactly the same manner prior to the onset of IB, the majority of kittens in the anticipatory stimulation group increased breathing activity following stimulation and increase in FiO_2 while most of the kittens in the reactive stimulation group became apneic despite the increase in FiO_2. Furthermore, although the reactive stimulation group received a 5-fold larger stimulus amplitude, we found that it was less effective than the gentler stimulation that was applied in anticipation of apnea. This could indicate that central processing of these stimuli is rapidly modified as the period of IB increases, where the gradually increasing level of hypoxia blocks or modifies somatic inputs arising from tactile stimulation,[22] thereby impairing the resumption of breathing and resolution of apnea. Indeed, it is well established that a fetus/newborn gradually stops breathing and becomes bradycardic, but also loses tone and becomes unresponsive to tactile stimuli, as hypoxia increases.[23,24] However, it is also possible that, although the stimulation location and vibratory frequency were the same, the difference in stimulation amplitude that we decided to use for the anticipatory and reactive stimulation triggered different sensory receptors or afferent inputs into the brain leading to potentially dissimilar responses.

It is important to note that in the reactive approach group, 2/11 kittens did not develop apnea and thus did not receive stimulation, despite developing an IB pattern that was similar to all other kittens. This type of biological variability is not unexpected and suggests that, in these cases, the respiratory center was able to stabilize breathing in response to the increase in FiO_2 without additional stimuli. Indeed, we would expect a similar number of kittens in the anticipatory approach group to have not required stimulation to prevent apnea. Although applying stimulation in the anticipation of apnea is likely to be considerably more effective, it is unknown whether this approach outweighs the possible adverse effects of unnecessary stimulation.

Anticipatory mechanical stimulation had a less pronounced and consistent effect on heart rate than breathing rate in this study. As there is a close link between the onset of apnea, hypoxia and bradycardia in preterm infants,[25,26] we had expected a stronger interdependency with regard to recovery. Our results might suggest that tactile stimulation primarily targets the respiratory centre and that recovery of heart rate is a secondary response which arises, for example, from a chemoreceptor mediated increase in oxygenation. Perhaps the kittens from our experiment remained sufficiently hypoxic to suppress their heart rate, even though anticipatory stimulation enhanced breathing.

The low variability in FRC during the experiment and the lack of difference in FRC between groups indicate that lung aeration, at this level, is not necessarily related to decreased or increased respiratory effort resulting from the change in FiO_2 or the presence or absence of stimulation. These results are consistent with previously published data showing similar lung aeration in two groups of preterm rabbits despite significantly different breathing rates resulting from different levels of FiO_2.[13] As we only included kittens that were breathing regularly at the start of the experiment, lung aeration had already occurred, as can be seen by the FRC levels measured. We have previously shown that within a few minutes of birth we would expect a FRC of 15-20 mL/kg in normal spontaneously breathing term kittens without CPAP.[27,28] However, with a CPAP 8 cmH_2O (initially 15 cmH_2O) our preterm kittens had FRCs of >20 mL/kg, which they were able to sustain even after the initiation of IB and apnea. Presumably, closure of the glottis (active adduction) in between breaths and during apnea helped to maintain FRC in kittens that developed apnea.[29] However, it is unclear how long the glottis stays adducted when apnea persists, because as the hypoxia deepens muscle tone and reflexes are lost.

To compare the effect of different stimulation approaches in this study, irregular breathing was induced by hypoxia. Although irregular breathing and apnea in preterm infants are not necessarily induced by hypoxia, using this approach gave us the opportunity to enforce a consistent starting point in all kittens within a comparable amount of time. It is unclear why the breathing rate appeared to decrease faster in kittens in the reactive group following the induction hypoxia, although there was no statistical difference between groups. It could have resulted from lower FRC values in the reactive group, which reduced their oxygen exchange capacity and increased the rate of decrease in oxygenation following the initial reduction in FiO_2. However, as FRC values are well within the expected range for both groups during the entire experiment, we would not expect this to significantly influence their response to stimulation. In addition, our study was limited by our inability to measure oxygen saturation in our kittens as our oximeter (Oximeter Pod ML320/F, AD Instruments, Sydney, Australia) is unable to read SpO2 values below 70% and so it is unknown whether the oxygenation level differed between groups. As the FiO_2 used to induce IB was similar and the FiO_2 provided in response to IB was exactly the same, we would expect that the oxygenation levels in both groups were similar at the onset of IB. However, as our results indicate that the degree of hypoxia impacts on the effectiveness of tactile stimulation, it would be desirable to include oxygen saturation measurements in further studies.

In conclusion, we confirmed that mechanical vibratory stimulation can prevent apnea and stimulate breathing effort in preterm rabbit kittens after birth. Timing proved to be a key factor in the effectiveness of stimulation, which is more successful when apnea is imminent rather than present. Based on our results it appears possible that anticipated automated tactile stimulation can improve current clinical care, provided that the benefits outweigh the possible drawbacks. The challenge of improving apnea treatment in preterm infants with automated tactile stimulation depends on finding the right balance between enhancing excitatory inputs and attenuating inhibitory inputs into the respiratory center while limiting the interference with other regulatory processes in the brain. However, anticipated stimulation can only exist in combination with yet to be developed predictive algorithms for impending apnea. Thus far, these algorithms have only been studied using pre-recorded physiological data.[30–32] and have not been evaluated in combination with a tactile response. Further studies are warranted to define the most beneficial closed-loop strategy for providing tactile stimulation to treat apnea of prematurity.

DATA AVAILABILITY

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

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AUTHOR CONTRIBUTIONS

SC, AP, and SH made substantial contributions to conception and design of the study. SC, JD, MC, KL KC, EM, TM, AF, MT, MW, MK, SH and AP performed experiments and obtained data. Data was analyzed and interpreted by SC, JD, MC, MK, SH and AP. The first version of the manuscript was drafted by SC, JD, SH, and AP. All authors are acknowledged for their critical revision of the manuscript and approval of the final version.

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COMPETING INTERESTS

The authors declare no competing interests.

INFORMED CONSENT

All animal procedures were approved by the SPring-8 Animal Care and Monash University's Animal Ethics Committees. Informed consent was not required.

ADDITIONAL INFORMATION

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