Protocol of controlled odorant stimulation for reducing apnoeic episodes in premature newborns: a randomised open-label Latin-square study with independent evaluation of the main endpoint (PREMODEUR)

Patricia Duchamp-Viret,1,2 Huu Kim Nguyen,3,4 Delphine Maucort-Boulch,5,6 Laurent Remontet,5,7 Aurore Guyon,8 Patricia Franco,9,10 Andrei Cividjian,9,10 Marc Thevenet,1,2 Jean Iwaz,5,7 Sonia Galletti,7,11 Behrouz Kassai,7,11 Elise Cornaton,3 Franck Plaisant,3 Olivier Claris,3 Hélène Gauthier-Moulinier3

ABSTRACT

Introduction Apnoea affects 85% of premature infants under 34 weeks of age and would be an important risk factor for subsequent neuropsychological disorders. Currently, premature children with life-threatening apnoea receive stimulants such as methylxanthines (mainly, caffeine) or doxapram (an analeptic unlicensed in children under 15). However, these products have undesirable effects (hyperarousal, irritability, sleep disorders, tachycardia) and are not always effective because apnoea does persist in some premature newborns. Previous studies have indicated that odorant stimulation, a non-invasive intervention, may stimulate the respiratory rhythm. The objective of the present protocol is to reduce the occurrence of apnoeic episodes in premature newborns by controlled odorant stimulation added to current pharmacological treatments.

Methods and analysis The project is a randomised open-label Latin-square trial with independent evaluation of the main endpoint (PREMODEUR). It will include 60 preterm neonates from two university hospital neonatal intensive care units over 2 years (2021–2023). Each newborn will receive no (S0), sham (S1) or real olfactory stimulation (S2) in random order. During S2, three distinct odorants (mint, grapefruit and vanilla) will be delivered successively, in puffs, over 24 hours. Mint and grapefruit odours stimulate the main and the trigeminal olfactory pathways, whereas vanilla odour stimulates only the main olfactory pathway. A statistical analysis will compare the incidence of apnoeic episodes during S1 versus S2 using a mixed effects Poisson model.

Ethics and dissemination Ethical approval was obtained from the Comité de Protection des Personnes Île-de-France XI (# 2017-A013-50-53). The results will be disseminated through various scientific meetings, specialised peer-reviewed journals and, whenever possible, posted on appropriate public websites.

Trial registration number NCT02851979; Pre-results.

INTRODUCTION

Sensory abilities of newborns

Newborns were long considered immature, almost deaf and blind and insensitive to odours or pain. Today, it is widely recognised that newborns have peripheral and cognitive abilities in sense organs and brain centres.1,2 The olfactory sense is, in particular, fully functional in newborns3; moreover, the spatial extent of the olfactory mucosa (or peripheral...
olfactory organ) is clearly greater in fetuses aged 5–9 months than in adults. From a functional point of view (sensitivity and olfactory analysis), newborns seem not only capable of detection and discrimination but also of habituation, memory and even learning. This olfactory sensitivity was found in premature infants as early as 28 weeks of gestation. Through facial mimics, newborns have expressed spontaneous preferences or disgusts for certain odours whose hedonicity seems rather universal and innate, particularly odours of vanilla (pleasant) or butyric acid (unpleasant). The perception of these odours do not only induce facial expressions and mouth or limb movements but also changes in breathing frequency.

Apnoea in premature newborns

Apnoea affects 85% of premature infants under 34 weeks of gestation. Clinicians consider that apnoea of prematurity is an important risk factor for subsequent neurological and psychological disorders. Indeed, in these frail infants, apnoeas may cause cerebral hypoxia that would lead to neuronal death with varying degrees of impact on brain development. Moreover, repeated apnoeic episodes are generally associated with altered neuro-motor prognosis at age 3 years.

Premature infants with apnoeas receive pharmacological treatments with stimulants such as methylxanthines (caffeine) or doxapram; but, in France, only caffeine has a marketing authorisation for premature newborn treatment. Nevertheless, both treatments have side effects (hyperarousal, irritability, sleep disorders, tachycardia, etc) and are not always effective because apnoea does persist in some premature infants despite treatment.

Apnoea and newborn’s sleep

Physiologically, sleep cycles do not proceed in the same way at all ages. Rapid eye movement sleep constitutes nearly 25% of total sleep time in healthy adults versus 80% in fetuses. This part decreases to 50% after birth, then to 33% at age 3 years and then again to 25% between age 10 and 14 years. In both humans and animals, apnoea induces, via hypoxia, an arousal reflex and increases the respiratory rate. In contrast, apnoea (thus, hypoxia) would not increase the respiratory rate in premature infants. This would be also true in newborns with congenital central hypventilation syndrome or newborns exposed to intrauterine nicotine. The same was seen in mice and ewes exposed to long periods of hypercapnic atmosphere at birth. Sometimes, in premature or frail newborns, apnoeas are not physiologically regulated, which is a risk factor for vital prognosis and/or neurological maturation. Moreover, in premature infants, this non-response to hypoxia may be favoured by excess heat in the incubator.

Odour perception and cardiorespiratory functions

In healthy adults, odours can be perceived during sleep. This perception elicits behavioural reactions that influence sleep quality; without awakening, odours may change the respiratory rate and raise consciousness level. Depending on the odorant, opposite effects may be exerted on the autonomic system. For example, grapefruit odour would be stimulating because it increases the cardiorespiratory rhythm, whereas lavender odour would be relaxing. The smell of grapefruit (or lemon, its main component, 95%) increases sympathetic nerve function and blood pressure (for up to 10 min after the end of odour stimulation) but decreases vagus nerve activity. This action on the vagus nerve could be of particular interest in newborns. Indeed, an overexpression of muscarinic cholinergic receptors as well as hyperactivity of cholinesterase were found in newborns who succumbed to sudden infant death syndrome. Those factors may have led to vagus nerve overactivity favouring sudden infant death.

A preliminary assessment of the effect of odours on the occurrence of apnoea was carried out in newborns resistant to pharmacological treatments. The study consisted in an uncontrolled ‘permanent’ odourisation (quality, intensity and dispersion) of an incubator with drops of vanillin adsorbed on newborns’ pillows. It showed a decrease in the frequency of apnoea (with or without deep bradycardia) over 24 hours of observation and no negative side effects. Indeed, during odorant diffusion, cardiorespiratory parameters remained stable as well as intestinal tolerance (vanillin did not significantly increase regurgitation). At the behavioural level, the authors reported no increased activity or excitability throughout the trial. More recent studies confirmed the beneficial effect of vanilla through reduction of the occurrence of apnoea.

Within this context, the aim of the present trial is to find out whether controlled odorant stimulation (as a non-invasive entry pathway that would stimulate the respiratory system) would reduce the occurrence of apnoea in premature newborns. The present article presents the trial protocol and details a method of controlled odorant stimulation that would supplement current pharmacological treatments.

OBJECTIVES

Primary objective

The primary objective is to determine whether controlled odorant stimulation (as a non-invasive entry pathway that would stimulate the respiratory system) would reduce the occurrence of apnoea in premature newborns. The present article presents the trial protocol and details a method of controlled odorant stimulation that would supplement current pharmacological treatments.

Secondary objectives

The secondary objectives are:

► Investigate the influence of the following factors on the effectiveness of odorant stimulation: the order of stimulation, gestational age, concomitant treatments that may influence respiratory function and their dosages.
► Determine whether the number and duration (mean and max duration) of apnoeas under real olfactory stimulation differ from the number and duration of apnoeas under sham or no stimulation.

► Evaluate the tolerance to real olfactory stimulation.

METHODS AND ANALYSIS
Experimental design

Patient and public involvement
No patient’s parent(s), legal guardian(s), or other public representatives have been involved in the design of this protocol.

Type of intervention
The study will be a randomised, open-label, Latin square trial with independent assessment of the primary objective.

Three test modalities will be used: (1) S0: no stimulation; (2) S1: sham stimulation during which only non-odourised medical air will be delivered; and, (3) S2: real olfactory stimulation with three odorants (mint, grapefruit and vanilla) delivered in the same order for all subjects and in same flow rate and same stimulation frequency as for S1.

The simulation sequence (each S0, S1 and S2 set) will be determined using a Latin square according to Williams’ scheme. This allows taking into account the carry-over effect. Six distinct sequences will be used: S0-S1-S2, S0-S2-S1, S1-S0-S2, S1-S2-S0, S2-S1-S0 and S2-S0-S1.

The newborns will be therefore exposed to three 24-hour stimulations (random succession of S0, S1 and S2). Each stimulation will be followed by a 24-hour washout period. The order of the three test modalities will be randomised and each newborn will be his/her own control.

A monitoring of a maximum of 3 days will search for any disorder (eg, infection) likely to interfere with the effects of the stimulations.

Number of subjects and study duration
Eighty newborns will be recruited to have 60 evaluable subjects (10 per specific sequence as detailed above). The premature newborns with randomised sequence will undergo cardiorespiratory recordings for five successive 24-hour periods (including washout) to determine the influence of odorant stimulation on the frequency of apnoea.

The whole recruitment period is expected to last 24 months. The duration of participation of each newborn will be a maximum of 12 days: 4 days for screening and selection, 5 days for experimentation (3 days for stimulation modalities + 2 days for in-between washout) and 3 days for medical supervision. The analyses and completion of the study report will require 6 months. Therefore, the duration of the project will be 36 months and 12 days.

Criteria for result evaluation

Main assessment criterion
The main assessment criterion will be the number of apnoeas or breathing pauses per 24 hours. This number will be obtained by continuous recording of the cardiorespiratory parameters via RECAN software: raw ECG waveforms, plethysmography (SpO₂—saturation of peripheral oxygen—sensor) and respiratory rate (impedancemetry with ECG electrodes). The averages of heart rate, SpO₂ and respiratory rate will be computed each second by the monitor. A specific graphical interface will detect events with a posteriori changeable threshold followed by manual validation. A pathological apnoea episode is defined either as complete cessation of breathing for more than 20 s (simple apnoea) or apnoea of any duration associated with bradycardia (heart rate <80 beats/min).

For each newborn, the study will look for a change in the number of apnoeas under real olfactory stimulation (S2) versus sham stimulation (S1).

Secondary assessment criteria
The secondary evaluation will include assessing:

► The changes in the number of apnoeas under real olfactory stimulation (S2) versus sham stimulation (S1) in function of the order of stimulation modalities, the gestational age, the concomitant treatments that may influence the respiratory function and their dosages.

► The difference between the number and duration of apnoeas under real olfactory stimulation (S2) and the number and duration of apnoeas under sham (S1) or no stimulation (S0).

► Tolerance parameters: adverse events and serious adverse events.

Evaluation of the benefit–risk balance

The main risk expected is an unfavourable change in respiratory rhythm with increase in apnoea episodes. This risk is deemed extremely limited; it has not been reported in previous studies that used odorants in neonates.\textsuperscript{7-10,34,35}

The expected benefit is being able to suggest a method of olfactory stimulation that decreases the number of apnoeic episodes. This non-invasive and non-pharmacological method will complement existing treatments (eg, routine caffeine) and, in the long term, would replace other treatments that have shown adverse effects. In a broader framework, olfactory stimulation would participate in the maturation of the autonomous nervous system of premature newborns as well as the maturation of limbic and cognitive brain structures. The benefit–risk balance of this project is expected to be positive.

Participants

Inclusion criteria
The inclusion criteria will be the following:

► Girl or boy born before 33 weeks of amenorrhoea from single or multiple pregnancy
Non-allergy to olfactory stimulation: coughing, sneezing, need for respiratory assistance likely to limit odour perception (continuous positive airway pressure or invasive assisted ventilation).

Signed informed consent obtained from the newborn’s parent(s) or legal guardian(s) by a neonatologist or a senior neonatal nurse after a 2-day reflection period.

Exclusion criteria
The exclusion criteria will be the following:
- Presence of severe congenital malformation.
- Suspected or confirmed central congenital hypoventilation syndrome.
- Need for respiratory assistance likely to limit odour perception (continuous positive airway pressure or invasive assisted ventilation).
- Allergy to olfactory stimulation: coughing, sneezing, tearing, respiratory arrest.
- Non-affiliation to the social security regime (for legal reasons).

Retention conditions
Participation will be proposed to all parents or legal guardians of eligible neonates. The participants will be followed-up as long as their stay in the neonatal intensive care unit (NICU). The unit staff will be called to solicit participation before any transfer of eligible newborn from the unit and prevent unnecessary transfers before completion of the protocol.

In case of enrolment problems, amendments to the protocol will be introduced to widen the age range.

In case of intervention discontinuation, no further data for the study will be collected, except the reason for discontinuation. Whenever necessary, the participant’s health status at NICU or hospital discharge might be later retrieved.

Discontinuation criteria
The intervention in any participant will be discontinued on appearance of any abnormal sign likely to be attributed to the use of the odorants (allergy, respiratory signs, neurological signs, etc). The study may be discontinued in case of safety problems on decision of the investigator and or the Data Safety Monitoring Board.

Whenever changes to the protocol are necessary, an amendment file will be constituted and transmitted to the Agence Nationale de Sécurité du Médicament and to the trial centres in order to update the protocol.

The Centre Régional de Pharmacovigilance (Lyon, France), a public and independent centre, will examine all cases of suspected adverse reactions.

Randomisation criteria
Only newborns who meet all of the following criteria will be randomised; the others will be excluded before randomisation.
- Newborns born before 33 weeks of amenorrhoea and aged more than 10 days.
- Presence of pathological apnoeas; precisely, children with at least three pathological apnoeas per day over three successive days as assessed by continuous RECAN monitors prior to randomisation (recordings reviewed by experts from the department of paediatric sleep disorders).

Random allocation method (randomisation)
Randomisation will be carried out after checking all inclusion and exclusion criteria and completion of the informed consent process. Randomisation will be centralised and use a dedicated internet server.

The randomisation list will be generated by the study statistician who will keep a copy. Randomisation will be carried out according to the plan defined by the Latin square: newborns will be randomised into six groups (at least 10 newborns per group) that correspond to the six possible combinations of S0, S1 and S2. The randomisation will be also stratified by centre (Lyon and Saint-Étienne). The date of randomisation will be the starting point of the study (date D0). The assigned treatment will start on the very day of randomisation.

Blinding
No concealment nor blinding are needed because: (1) each participant will be his/her own control; (2) the newborns are not supposed to be influenced by the randomisation process; and, (3) healthcare members who carry out the experiments do not participate in data processing or analysis.

Interventions
Products under study
The trial will use distinct odorant stimuli to avoid the phenomenon of sensory habituation to repeated stimulation with the same odorant likely to lead to decreases in behavioural and cardiorespiratory responses. Moreover, the odorants should be unfamiliar to the newborns (eg, breast milk) because of possible unspecific reactions according to hunger or satiety.

In newborns, even premature ones, the main and trigeminal olfactory systems are already functional. The trigeminal system makes it possible to treat somatosensory sensations caused by odorant molecules (fresh, hot, pungent, odorant, etc.). Detection is carried out by the free endings of the sensory fibres of the ophthalmic branch of the trigeminal nerve that innervate the face, the scalp and the oral and nasal mucosa. The choice of odorants that stimulate the trigeminal system should compensate for possible lower olfactory stimulation linked to the use of a high-flow intranasal cannula that reduces the openings of the nostrils. However, the choice
of odorants that stimulate specifically the olfactory system (like vanilla used by several authors\textsuperscript{9,34,35}) will be also important because of the hypothesis of alteration of the trigeminal nucleus in newborns who succumbed to sudden infant death syndrome.\textsuperscript{37} Thus, the odorants chosen should stimulate both the main and the trigeminal olfactory systems to compensate for possible failure of any of them.

Moreover, in humans and newborn mammals, some odorant molecules acting on both the main and the trigeminal systems have been reported to influence the cardiorespiratory rhythm,\textsuperscript{26-31} especially during sleep, and thus, should influence apnoea in newborns.

Accordingly, ‘vanilla’ seems to be an odorant of choice because of its known effects on apnoea.\textsuperscript{9,34,35} ‘Mint’ and ‘citrus’ are also very interesting odorants because of their effects on the cardiorespiratory rhythm.\textsuperscript{28-31}

Finally, the use of pure molecules seems essential to ensure stimulus consistency over time (constant volatility and composition of the vapour phase).

According to all those conditions, the molecules chosen for the trial will be: (1) (R)-(−)-Carvone, CAS RN 6485-40-1 (herein written CAR); a spearmint scent that stimulates the olfactory and the trigeminal system (food grade, Sigma-Aldrich, France); (2) Ethylvanillin, CAS RN 121-32-4 (herein written EVAN); a vanilla scent that stimulates the olfactory system (food grade, Sigma-Aldrich, France); and, (3) (R)-(+)−Limonene, CAS RN 5989-54-8 (herein written LIM); a grapefruit scent that stimulates the olfactory and the trigeminal system (food grade, Spectrum, USA). CAS is the product registry number in the Chemical Abstracts Service database.

Preparation and handling of trial products

Odorants in liquid phase (CAR and LIM) or powder phase (EVAN) will be adsorbed to PEBAX 33 MED SERIES beads (Arkema, France). The transfers to PEBAX beads will be as follows: 5 g (±0.1 g) of PEBAX beads will be packed in each of three 40 mL plastic vials and each vial will receive either 2 mL of LIM, 2.5 mL of CAR or 0.4 g of EVAN, then shaken to disperse the liquid or the powder on the beads. Each of these preparations will be kept at room temperature and away from light for a minimum of 24 hours to ensure total adsorption. The three vials of odourised beads will be transferred to three U-tubes closed with Teflon screw caps: the so-conditioned odourised beads can be stored for 30 days without risk of instability.

All preparations will be carried out by in a single laboratory and the storage made at the pharmacy for indoor use (PIU) of each centre until randomisation and use. The batches of the study products will be labelled by the preparing laboratory according to the regulations and sent for use in the NICU. The dispensing will be nominative and carried out by each centre’s PIU. The dispersed batches will be traced per newborn. Unused batches will be returned to each centre’s PIU for recycling or destruction.

Odorant administration

Each of the odorant will be diffused in gas phase using a portable olfactory stimulator or ‘olfactometer’ (figure 1A). This device is a prototype dedicated to this trial and developed by CNRS UMR 5292 (NEODEUR: European patent N° 602017020 520.6 / FR3411104; delivered on 29 July 2020; Medical Device for Olfactory Stimulation). The olfactometer will be placed outside the incubator; only a Teflon tube whose end opens by a small plastic single-use funnel (figure 1B) will be placed inside the incubator. During administration, the funnel will be placed at nearly 10 cm from the newborn’s face in the axis of the nostrils. The ventilation flow of the incubator and the use of a high-flow nasal cannula will be taken into account. During S2, the three odorants contained in three U-shaped glass tubes (supplied by each centre’s PIU) will be connected to the olfactometer by an authorised investigator.

The olfactometer will diffuse the odorants with strictly controlled and reproducible parameters of quantity, duration and sequence. During olfactory stimulation (S2), the stimuli will consist of 5 s puffs of LIM, CAR or EVAN delivered successively at 5 min intervals and at a constant flow rate of 500 mL/min.
Figure 2 Schedule of a typical experiment. From the first day of screening (D-4) to the last day of medical surveillance (D7), the recordings will last 12 days for each newborn. The recording by RECAN will be reset to 0 at D0; the first day of the clinical trial. The trial will alternate, over 5 days, 24-hour periods of stimulation (S: either S0, S1 or S2 as assigned by randomisation among the six different possible combinations) and 24-hour period of washout (WO).

The functioning of the olfactometer underwent a 24-hour non-stop test in the same conditions as the present protocol. The odorant puffs were measured by a photoionisation detector; they showed consistency and reproducibility with the three molecules. During the experiments with the newborns, the vector gas that will extract the odorants from the PEBAX beads will be medical air to limit the transmission of pathogenic microorganisms. During sham stimulation (S1), the olfactometer will deliver medical air at same flow rate and frequency for 24 hours.

Clinical investigations
During each experiment, cardiorespiratory parameters will be continuously recorded over 8 days; for four consecutive days from D4 to D1 (pre-randomisation) and then for five consecutive days from D0 to D4 included (post-randomisation) of which 3 days of administration (S0, S1, S2 in random order) and 2 days of washout (WO1, WO2) (figure 2).

Cardiorespiratory parameters and stimulation times will be recorded over 24 hours a day and stored via RECAN software. This software is a data logger designed to acquire analogue and RS232 signals from different devices and collect continuous cardiorespiratory parameters over the 8 days. An RS232 link connects it to the olfactometer that sends a signal indicating the time, the duration and the nature of the stimulation in relation with the temporal courses of the cardiorespiratory events. This ensures an exact timing of each cardiorespiratory event relative to the test modalities. The programme is also able to record information from the newborn monitor alarms (via USB). An interface dedicated to the nursing staff allows them to write, in real time, comments concerning the alarms as well as the events likely to disturb the protocol such as gastric feeding, handling the newborn, changing the diaper, etc.

The reading and analysis of the recordings will be carried out by an independent evaluator who is an expert in the software and signal analysis. RECAN includes processing tools that allow defining multiparameter searches (threshold, frequency, co-occurrence of events, temporal and frequency analyses of the activity of the autonomic nervous system, etc). The analyses will determine changes in the occurrence of apnoeas under different experimental conditions (no, sham and real olfactory stimulation). In the case of an artefact on a tracing, the medical record completed by the nursing staff will allow deciding whether it was an apnoeic episode or not. This reassessment will be blinded to the modality received by the newborn. All recordings will be sent to a single centre (the Sleep Unit) as anonymised data on a USB flash drive.

Data and study management
Observation notebooks
All data required by the protocol will be collected with explanations for missing data. All clinical or paraclinical data will be transferred to electronic case report forms (CRFs) as soon as they are obtained. The statistical analysis will apply methods to handle missing data according to their nature and number.

The data will be available to the investigators, the trial physicians, the trial assistants, the biostatisticians and the members of the Data Safety Monitoring Board. The data confidentiality is governed by an official MR001 declaration and the European Union (EU) General Data Protection Regulation.

Source documents
Source documents will be the original data and records from which patient data will be transferred to the case report book. These will include (but not be limited to) test result reports, hospital temperature curves and/or medical notes, dispensing notes and medical correspondence.

The investigator will allow direct access to study source data during monitoring, audit or inspection visits. A copy of the CRF will be kept by the investigator for his/her own records. The investigator will keep all observations and the signed consent of the parents/legal guardians for a minimum of 25 years. The data of the study will be computerised in a coded way and in accordance with the Data Protection Act 2018 (EU General Data Protection Regulation). The CIC data management department (Centre d’Investigation Clinique, Lyon, France) will be responsible for the computer management of the data.

Consent forms and documentation given to the participants’ authorised surrogates are available on motivated request from the principal investigator.

Study management committees
The Coordination Committee will ensure the progress of the study; solve any problem related to its conduct; and decide on the study discontinuation, outcomes and publication. It will involve two neonatologists (of whom the principal investigator), three researchers and one nurse as trial assistant.

The Steering Committee will ensure the overall supervision of the conduct of the study according to the current recommended standards and practices, and prepare the
The number of subjects needed was calculated by simulation using a Poisson mixed-effects model, the model that will be used for the main analysis. Based on the raw data reported by Marlier et al a model was fitted to determine parameters that were subsequently used in a Monte-Carlo simulation. These parameters were the mean number of apnoeas under S1, under S2 and the interindividual variability of these numbers. Marlier et al reported a 36% reduction in the number of apnoeas. Because of the small size of that study (N=14), we considered the lower limit of the CI of this reduction; that is, 10%, to account for the large uncertainty around this variation. Thus, assuming a 10% reduction between S1 and S2, 60 individuals were deemed required to reach a statistical power of 90% with a 5% two-sided significance level. However, as the secondary objectives involve interaction testing, the number of subjects needed for these objectives should be higher: this number will therefore be the maximum recruitment capacity.

Some potential confounding factors such as caffeine treatment or maternal history of smoking during pregnancy will be mentioned in the CRF and taken into account in the final analysis.

The analyses below will be performed on an intention-to-treat basis. No interim analyses are planned because of the short duration of the whole study.

Trial conduct audits will be carried out by the Promoter. The first one will take place after the inclusion of the second participant then after inclusion of every group of 10 participants.

**Main analysis**

The main objective will be analysed by testing the effect of stimulation S2 versus S1 using a Poisson model with a random ‘newborn’ effect and retaining only the measures (ie, numbers of apnoeas) under sham and real stimulation. The basic assumptions of the Poisson model will correspond to testing whether ‘\( \beta_0=0 \)’, parameter \( \beta_1 \), reflecting the change in the number of apnoeas, at a given individual risk \( u_{ij} \), between a sham stimulation S1 and a real stimulation S2 (ie, \( \beta_1 \) corresponds to the ratio between the number of apnoeas under S2 and the number of apnoeas under S1, all other things being equal). This test will be a likelihood ratio test with type I error of 5%. If this test indicates no significant difference, it will be concluded that there is no effect of real stimulation (vs sham stimulation).

**Secondary analyses**

The first secondary objective will be analysed using a model similar to the one used in the main analysis but to which will be added two factors of interest: gestational age and concomitant treatments. The analysis will test the interactions between these factors and the stimulation effect as well as the interaction between factor ‘order’ and factor ‘stimulation’ as defined above.

The second secondary objective will be analysed in the same way as the main analysis. This analysis will test the effect of stimulation S2 versus S0.

**Discussion**

In newborns, prematurity, high proportion of paradoxical sleep (≥50%) and intrauterine exposure to nicotine (≥30%) are all factors that favour the occurrence of apnoeic episodes and may engage the vital prognosis. Exposure of newborns to odorants they could perceive without sleep disturbance would be an easy and noninvasive way of preventing or limiting apnoeic episodes by stimulating the entire cardiorespiratory system.

The use of odorant stimulation to drive the cardiorespiratory rhythm would involve the autonomic system via several putative olfacto-motor pathways. The existence of such pathways is supported by several animal studies. For example, in rats, novel odorants induced changes in sniffing frequency which becomes extremely rapid, advocating an olfacto-motor regulatory pathway. In rabbits, olfactory stimulation induces not only a rapid sniffing response, but also an excitatory discharge in the respiratory centres of the brain stem.

In humans as in animals, the hypothesis is that olfactory stimulation acts from the olfactory bulb on the modulating respiratory centres via different cerebral pathways passing mainly through the hippocampus (for a review see) where neurons discharge in rhythm with respiration; thus, the olfacto-motor pathway may not involve the hippocampal relay. In mice, starting also from the olfactory bulb, one pathway would involve the amygdala and then the parabrachial nucleus (belonging to the pontomedullary structures) which is thought to play an important role in cardiorespiratory control, whereas another pathway would go to the cerebellum via the ventral tegmental area.

Current pharmacological treatments used to reduce the frequency of premature newborn apnoeic episodes are debatable. In comparison, olfactory stimulation
is much less invasive and quite easy to implement. It would complement existing treatments (eg, caffeine) or replace other treatments with potential side effects (eg, doxapram). Besides, odorant stimulation in premature newborns is likely to contribute to the maturation of the bulbar respiratory centres, the autonomic nervous system, the limbic system and the cognitive areas because olfactory integration processes involve all these structures.

If odorant stimulation proves effective within the context of prematurity, it will be extended to the treatment of sleep apnoea in older children though this condition is not linked to immaturity of the respiratory centres as in premature newborns. Indeed, premature born children may be more often subject to sleep respiratory disorders and present obstructive apnoea in childhood. Furthermore, because, it stimulates the feeding centres, odorant stimulation may also be used to promote oral feeding in premature newborns.

As olfactory stimulation seems to be a non-invasive way of influencing the sequences of different cycles as well as the quality of sleep, it may be used in fundamental research in sleep physiology, treatment of diseases related to sleep apnoea or the control of other conditions requiring cerebral sensory awakening (particularly, dysphagia in the elderly through maintaining the swallowing reflex, or even coma).

This trial was motivated by the seriousness of sleep apnoea in neonatology and the extents of the therapeutic fields of olfactory stimulation. Assessing objectively and accurately the benefits from the approach requires a strict control the olfactory stimulation (nature, quantity, duration and sequence). This will be satisfactorily ensured by an olfactometer or ‘portable odorizer’, a prototype specifically designed for the present protocol to allow a perfect control of odorant diffusion in the gas phase. The validation of this olfactometer in a clinical study will offer development opportunities for the design of olfactometers for medical or other specific purposes.

Among the potential limitations, one could imagine an insufficient number of included newborns, group imbalance due to caffeine treatment or another unexpected source of bias or a reduced effectiveness of odorant stimulation in premature newborns with the high flow nasal cannula because of the partial obstruction of the nostrils by the device.

In case of success, one potential immediate benefit from the method will be a non-invasive and non-pharmacological solution to apnoea in premature newborns and a convenient way to stimulate the maturation of their autonomous nervous system and even cognitive functions.

Author affiliations
1Centre de Recherche en Neurosciences de Lyon, Bron, France
2Équipe de Neuro-Éthologie Sensorielle, UMR 5292, Bron/Saint-Étienne, Rhône-Alpes, France
3Service de néonatologie et de réanimation néonatale, Hospices Civils de Lyon, Hôpital Femme Mère Enfant, Bron, France
4Département de Pharmacotoxicologie, Hospices Civils de Lyon, Lyon, France

Acknowledgements The authors thank the nurses, the physicians and the head of the neonatal intensive care units of Hôpital Femme Mère Enfant for their involvement in the design of this trial. They also thank Christian Delvar, the engineer who developed the software of the olfactometer used for this study.

Contributors Study conception and design: PD-V, HGM, HKN, DM-B and LR. Study coordination: PD-V, HGM, HKN, SG, BK, OC and PF. Mechanical and pneumatic design of the olfactometer: TM, PD-V. Data collection: HGM, HKN, SG and EC. Data management: HKN, AF, GP, SG and AC. Data analysis: DM-B, LR and JI. Manuscript drafting: PD-V, HGM, HKN, OC and JI. Final manuscript revisions: All authors.

Funding This study will be supported by the Direction Générale de l’Offre de Soins (DGOS) (PHRCI - 2017).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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ORCID iD Patricia Duchamp-Viret http://orcid.org/0000-0002-7730-266X

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BMJ Open: first published as 10.1136/bmjopen-2020-047141 on 13 September 2021. Downloaded from http://bmjopen.bmj.com/ on September 18, 2023 by guest. Protected by copyright.


