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Editorial

The Development of Oral Feeding Skills in Infants

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Appropriate nutritional intake is a major component of growth in infants. Interests in nutrition customarily have been centered on the types of nutrients and caloric intake offered, for example, the benefits of mother's milk over that of formula, presence/absence of growth factors, and potential advantages provided by probiotics early in life [1, 2]. An important component of infant nutrition that has been overlooked until recently is the ability of infants to take their nutrients by mouth safely and successfully. As the majority of healthy term newborns are readily taken to the breast or bottle soon after birth, the ability to feed by mouth generally does not raise concern. However, over the last two decades, health professionals along with families of infants born prematurely have come to realize that a great number of these infants, notwithstanding the type of milk taken (mother/donor milk, formula), cannot readily feed by mouth which puts them at risk of adverse events ranging from oxygen desaturation to aspiration pneumonia [3]. Long-term oral feeding difficulties resulting from such early incompetence have also been identified through the increased feeding disorders clinics that follow these infants [4–6]. Unfortunately, basic knowledge regarding the development and physiology of infant oral feeding skills is still lacking.

No medical events solely impact on a patient's condition [7]. This is particularly true with infants who are helpless and must rely on caregivers, particularly their mother, for survival. Consequently, infant's growth and development become a function, not only on their own maturing attributes and their surroundings, for example, neonatal intensive care unit or home environment, but also on the quality of their interactions with mother/caregivers during difficult times. If feeding difficulties persist, consideration of the quality of interactions within the mother-infant dyad must be taken into account.

Figure 1 is an attempt to summarize the complexity of this paradigm.

This special issue presents some of the latest clinical and basic research concerns in this area, but is by no means representative of the intricacies of the above model. Each of these studies addresses a particular piece of the puzzle. However, if safe and successful oral feeding is of primary concern when working with infants, it is essential to keep in mind that multiple factors can lead to the same adverse outcomes, rendering the identification of the primary causes difficult.

N. Bertoncelli et al. presented a summary review of our current understanding of bottle feeding competence in healthy preterm infants. A. Jenik et al. addressed one of the most common clinical issues experienced by preterm infants when transitioning from tube to independent oral feeding, namely, hypoxic episodes during bottle feeding. However, based on the above oral feeding puzzle, pulmonary immaturity/insufficiency ought not be systematically presumed as the culprit. S. M. Barlow et al. noted that frequency modulation and spatiotemporal stability of nonnutritive sucking bursts were differentially expressed in infants with and without respiratory distress syndrome. As these measures

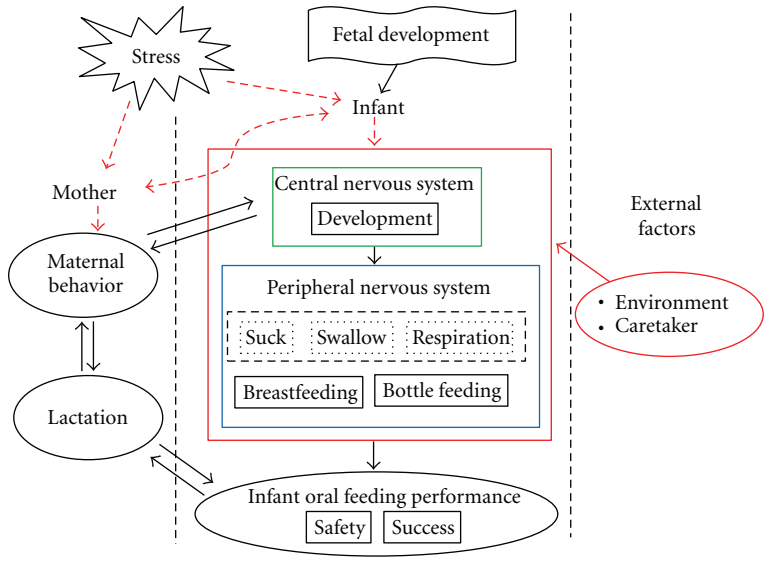

Figure 1: Oral feeding puzzle.

are indicative of the "steadiness" of the suck central pattern generator, the authors suggested that alteration in these measures may potentially be used to gauge the developmental status and progression of oromotor control systems in infants.

Although breastfeeding is acknowledged as the optimal feeding mode, its research and understanding lag behind that of bottle feeding. This may be due to the fact that the study of infant's skills during bottle feeding can be achieved more readily and accurately than during breastfeeding. Indeed, the maternal component associated with milk availability/release has a direct impact on the feeding performance of an infant which is not present during bottle feeding. It is generally claimed that infant sucking skills differ between bottle- and breastfeeding and thus information gathered from bottle feeding is not necessarily applicable to breastfeeding. For instance, although appropriate coordination of suck-swallow breathe is critical for safe oral feeding, incoordination of these three functions results in different outcomes between these two feeding modes. Consequently, findings cannot be extrapolated so readily from bottle to breastfeeding [8, 9]. V. S. Sakalidis et al. in this issue is one of few articles suggesting that similarities can be found between these two modes of feeding, namely, that the use of vacuum (the suction component of sucking) by infants may be equally conducive to safe and coordinated milk removal be they breast- or bottle-feeding. As studies have shown that bottle-feeding infants can modify their sucking pattern based on the rate of milk flow [10], it is conceivable that the differences alluded to between breast- and bottle-feeding lie primarily in maternal milk release during breastfeeding. Mother's milk is acknowledged as the optimal nutrition due to its nutritional and immune factors. However, there is evidence that maternal diet during lactation may be a route for allergen exposure potentially resulting in infant's sensitization. The study of J. Paton et al. continues to support such speculation.

J. L. Maron's study introduces the exciting notion that the developmental expression of key regulatory genes may play a role in successful oral feeding development, in particular those associated with feeding behavior, cranial nerve development, and the development of the nervous, skeletal, and muscular systems. Potential genetic involvement in the field of infant oral feeding has not yet been well acknowledged.

The remaining articles by J. R. Alberts and R. Pickler, J. R. Alberts and A. E. Ronca, C. Ayres et al., N. Reissland et al., and M. Trabalon and B. Schaal take a fresh look at the impact that fetal and postnatal development may have not only be on the mechanistic determinant(s) of infants' success at oral feeding, but also on their role as potential players in the development of postnatal physiologic and behavioral functions associated with feeding, such as sensory (e.g., gustatory, olfactory), neuromotor, and digestive processes. They provide support for the concept of Anokhin's developmental model of "Systemogenesis" that relates to the heterochroneous maturation of physiologic systems that a newborn organism needs to undergo to optimize its survival and successful adaptation to its ex utero environment [11, 12]. Indeed, they highlight how perinatal organisms integrate phylogenetic and ontogenetic neuro-behavioral antecedents to direct their neonatal abilities to cope with the adaptive challenges imposed by their typical, as well as atypical environments. It is hoped that these studies will sensitize clinicians and researchers towards the plasticity and limitation of neonatal oral abilities and encourage further research to characterize the sensitive periods during which the various elements of oral feeding skills develop, such as sucking, swallowing, and coordination of suck-swallow respiration.

In summary, the articles presented here illustrate the many causes that can lead to oral feeding difficulties. This special issue contributes to the stimulation of the emergence of innovative research questions and the increased

interdisciplinary collaboration between clinicians and basic researchers. The oral feeding puzzle emphasizes the importance that maternal and environmental factors can have on the outcome of the infant. A deeper understanding of the many causes at play and the development of efficacious preventive and therapeutic approaches will advance the care of infants with oral feeding difficulties.

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Review Article

The Experience of Being Born: A Natural Context for Learning to Suckle

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Understanding the developmental origins of congenital capabilities such as sucking is fundamental knowledge that can contribute to improving the clinical management of early feeding and facilitate the onset of oral ingestion. We describe analyses in rats showing that sensory stimulation *in utero* and during birth establishes the newborn's sucking responses to maternal cues. We mimicked elements of labor and delivery (viz., compressions simulating labor contractions, stroking simulating postnatal maternal licking of the newborn, and postnatal thermal flux), and used them to induce postnatal respiration and nipple attachment in caesarian-delivered pups. We report herein new data showing that, by simulating a fetal rat's experience of being born, specific components of vaginal birth provide sufficient conditions for the odor learning that guides newborn's sucking responses. In contrast, the absence of *in utero* compressions was associated with poor sucking onset. Knowing how birth stimuli contribute to the first nipple attachment and constitute a context for learning to suckle is an important step toward better management of some early feeding problems. It can serve also as a foundation for understanding the challenges of facilitating sucking by babies born prematurely so that they do not experience the typical contingencies mediating onset of oral ingestion.

1. Introduction

The ability of a newborn baby, fresh out of the womb, to attach to the maternal nipple and begin sucking leads many to label the behavior as "innate." Some extend this concept of innate behavior to include reflexes, denoting fixed action patterns organized as sensory-motor circuits in the brain stem or spinal cord [1, 2], as well as the rhythmic firing of "central pattern generators" (CPGs), also located in the central nervous system, that produce correlated neuromuscular sucking rhythms [3].

The concept of "innate" behavior is controversial, to say the least [4, 5]. Criticisms abound because such behaviors, when carefully observed and measured are not fixed but are highly variable [6, 7]. Moreover, so-called "instinctive" behaviors, under the scrutiny of experimental analysis, prove to be based on various forms of prior experience and learning [8, 9]. Similarly, while the firing of CPGs may correlate with sucking rhythms, it has yet to be shown that such isolated elements actually combine with other discrete components to create the real behavior of the suckling newborn, behavior that adapts to the unpredictable, dynamic geometry of the mother's body, and behavior in real time and in real contexts [6].

Congenital is a more accurate and defensible term with which to denote a capability in behavior that is present at birth. In contrast to "innate" and "instinctive", congenital is more obviously a *description* of status at birth than it is an *explanation* of its basis or origins [10, 11]. Explaining the developmental origins of a congenital capability such as nipple attachment and sucking by a newborn upon confronting for the first time its mother's external body in highly novel environment is a formidable challenge of clinical significance.

The experimental literature, based on studies with nonhuman animals, contains a wealth of information pertinent to a better understanding of the onset and development of oral feeding skills. This literature includes some impressive findings concerning the onset of nipple attachment and sucking by newborn rat pups.

A brief description of the postnatal onset of sucking by rat pups will help frame the forthcoming presentation and analyses: rat pups are born after a gestation period of about three weeks. They are born as litter, averaging 10 pups (mothers have 12 nipples), blind, deaf, and furless, with limited strength and coordination. For about 6 hrs prior to parturition, labor contractions can be seen rippling vertically on the mother's abdomen or indenting her sides as she stretches her body. More than 100 labor contractions can be observed during a rat's labor [12]. As each pup emerges from the birth canal, encased in an amniotic sac with the umbilical cord and placenta trailing, the dam assists with licking and gentle tugging. The mother removes the sac by licking and nibbling, consumes the placenta and membranes, licks the pup some more, licks herself, and then repeats the sequence as the next pup emerges. Only after all the pups are thus delivered and the placentas are all consumed, does the rat dam turn her attention to the newborns, which she gathers into a clump in the nest and settles over them.

We have described and quantified the labor and delivery process in rats, noting the stimulation received by the pups during labor and throughout the birth process [12]. In the nest, with the dam hovering above the pups, the infants are active. They orient to the dam's ventrum, probe against her body and root along the ventrum until they orally grasp a nipple and suck. It is difficult to observe directly the natural sequence of events that lead to the rat pups' initial nipple attachments and sucking. When the dam settles on her newly born litter she is typically crouched above the pups within a nest that affords poor visibility. Fortunately, the newborn rat's behavior is robust and orderly; when placed under suspended artificial surfaces simulating various properties of the dam's ventrum, newborn rats show an organized repertoire of behaviors. They travel, wriggle, turn on their sides and upside down, ventroflex, probe the surface, and audibly bark, all in a state of heighted behavioral arousal [13]. Other studies have been conducted with the dam anesthetized and the pup's behavior thus isolated for analysis. Thus, it is known that olfactory cues present on the rat mother's nipples and ventrum are necessary and sufficient for newborn pups to locate and orally apprehend a nipple to suck. These odor cues can be removed by washing the nipples and surrounding body surfaces that eliminates suckling [14, 15]. Nipple attachment and suckling by newborns can be reinstated, however, by painting onto the dam's ventrum a distillate of the wash taken from the dam's body or by painting nipples with amniotic fluid or maternal saliva [15]. Other substances, both natural and atypical were tried, but no others were effective in reinstating nipple attachment [15].

Knowledge that amniotic fluid is a sufficient stimulus to elicit the newborn's first nipple attachment led to preliminary considerations of two, mutually exclusive possibilities. One was that the key olfactory stimulus is somehow predetermined and that the newborn is correspondingly and inherently prepared ("hard-wired", so to speak) to detect and respond to the cue. The second possibility was that the perinate responds with nipple attachment and sucking to the amniotic odor stimulus because of its previous experience with amniotic fluid. Pedersen and Blass [16] translated these contrasting explanations into an experiment with newborn rats. They reasoned that if amniotic fluid is a behaviorally potent stimulus because the fetus experienced it previously, then if some other odor was similarly experienced, it should have the same behavioral potency as amniotic fluid.

They tested this bold hypothesis by adding a novel, lemon-like substance to the amniotic fluid, and then testing whether this chemical would rescue the newborn's ability to make its first attachment to the washed nipples of a mother rat. The previously validated experimental procedure [17] involved externalizing the uterine horns of a gestational day (GD) 20 dam and injecting a small quantity (0.2 mL) of a citral and saline solution through the transparent wall of the uterus into the amniotic fluid. The uteri were replaced in the dam's peritoneum, the laparotomy incision was closed, and gestation was completed without complications. Then, on GD 22, pups were delivered by caesarean section and placed immediately for one hr in a warmed nest where they were stroked with "a soft artist's brush" for 1 hr in the presence of the citral odor.

The test procedure involved presenting the caesarean-delivered pups with an anesthetized parturient rat dam (not the subject pups' mother). If the dam's nipples were washed, pups did not attach to nipples, but when citral was on the dam, the treated newborns attached to a nipple and sucked! The new odor elicited the sucking behavior but, remarkably, "natural" odors of an unwashed dam were not effective for the citral-treated perinates. The new odor had replaced the natural stimulus. They performed an additional experiment in which pups were exposed to citral (a) *in utero*, (b) immediately after birth with stroking, or (c) both *in utero* and with postnatal stroking. Only pups with the combined experiences attached to the washed, citral-scented nipples and not to the unwashed, normal nipples.

Pedersen and Blass' [16] study provided important new insights into the initial plasticity of the newborn rats' sucking, especially the specification of the cues that can activate and direct the behavior. Their findings created a host of new questions. It seems clear that the establishment of the olfactory control of sucking is determined by the experiences of the perinatal rat pup. But, *what are the essential experiences for establishing the newborn's sucking responses to maternal cues?*

We adapted elements of our previous investigations of the perinatal rats' sensory experiences in the uterine environment and of the birth process [12, 18–20] to demonstrate that specific components of maternal stimulation are sufficient conditions for the odor learning that establishes the newborn's sucking responses to maternal cues. The present paper is a review of some of this past research as well as a report of additional, previously unpublished data that, together, provide a new view of how the *experience of being born* creates a context for learning. That is, embedded in

flow of events that constitute the birth process are forms and levels of stimulation that, together, create the contingencies for early, rapid learning in the fetus, as it becomes a newborn. We will show that this learning, though general in initial form, is expressed in the natural context of the mother's body as organized, adaptive, seemingly goal-directed behavior.

We will first review an analysis of rat maternal behavior during labor and delivery from which we derived a set of novel tools that enabled us to simulate the major components of vaginal birth. We will also review some of our evidence that fetal and neonatal rats (perinates) have sensory capabilities sufficient to experience the birth process, at least the elements that are needed for basic associative learning. Then, we will present data showing that the perinates' responses to simulations of the birth process (a) augment nipple attachment and sucking, (b) establish odor-guided responses to the mother, and (c) induce neural conditions that mediate state transitions between fetal and neonatal behavioral systems which can account for the activation and expression of the newborn's initial sucking behavior.

Under laboratory conditions, Norway rats typically give birth on the 22nd day of gestation; by breeding our animals on a known day, we were able to be present with appropriate video arrangements to view and record the dams' labor and delivery [12]. From these videorecordings, we quantified an average of 144.6 labor contractions during the six hours prior to the birth of the first pup from eight dams. Figure 1 illustrates the three types of visible labor contractions in rat dams and shows the average frequency of each during the 6 hrs of labor. Behavioral expressions of labor in the rat progress from uterine peristalsis to lordosis contractions followed by vertical contractions that occur in close association with birth of each pup. The brisk, linear decline in interpup contraction intervals shown in Figure 2 indicates how the contractions quicken as parturition approaches.

From our systematic observations, we are able to describe the labor and delivery in the rat. Parturition in all 8 dams occurred within 12 hrs of E22. Duration of the delivery phase of parturition (first to last birth) ranged from 40 to 136 min, with dams delivering and average of 10.1 ($\pm$1.1) pups. Delivery duration and litter size were positively related ($r^2 = .73$, $P < .01$) across the dams.

As each pup begins to emerge from the birth canal, the dam typically adopts a head-between-heels posture, which facilitates delivery by enabling the mother to use her teeth to grasp the newborn and extract it from the vagina. Mothers lick and handle each pup, removing and consuming the embryonic membranes, activities that produce cutaneous stimulation and augmented evaporative cooling of the newborn's body. Dams provided about 2 min of continuous stimulation to each newborn while participating in its delivery.

Nearly all the pups were born in the vertex position. Thus, the initial stimulation from maternal licking was to the offspring's head. Each pup also received bursts of vestibular stimulation as the dam rotated its body while systematically consuming the products of gestation. During the initial phases of intense tactile and vestibular stimulation, pups were unresponsive. As birth membranes were removed, especially from the head, pups began to emit the robust gasps that are characteristic of the onset of independent respiration [21], and thereafter they displayed gross movements and audible vocalizations. After the immediate postpartum licking and handling of each newborn, the dam often refocused her attention on previous newborns, providing each one with about 2.5 min of additional licking and handling. Overall, licking by the dams was distributed relatively evenly across the pups' bodies: head (39%), body (24%), anogenital area (32%), (see [12] for additional details).

Such observations help to specify events to which the newborn rat is exposed during birth. Each pup received a protracted bout of repetitive tactile and vestibular stimulation associated with the dam's handling and licking. During parturition, offspring are exposed to seemingly harsh forms of stimulation related to cooling and with compressions under the weight of the dam's body [12].

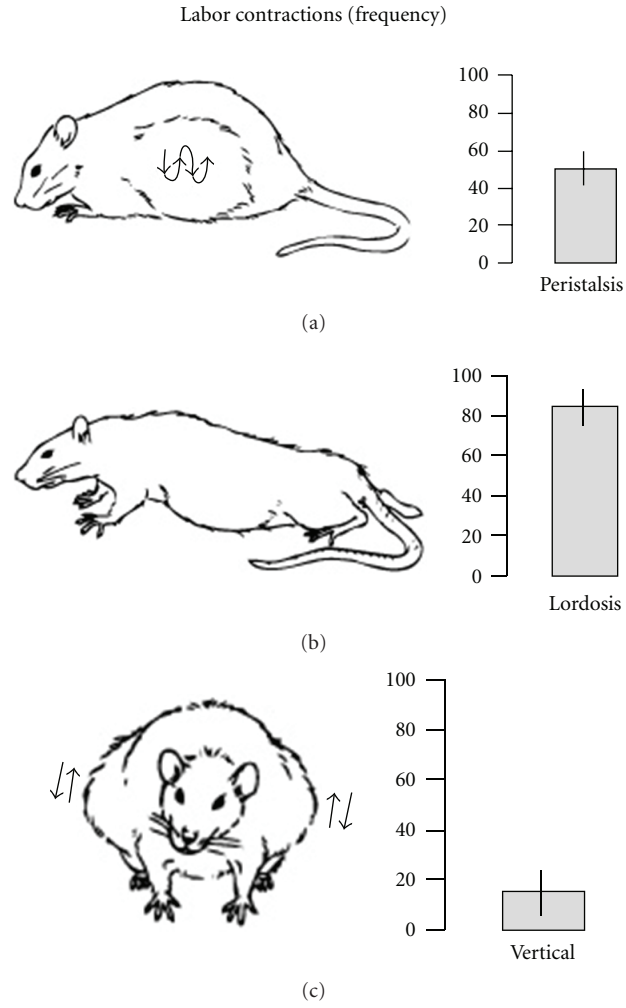

Figure 1: Frequency of three types of labor contractions observed in Norway rats. Histograms show mean frequency of peristalsis, lordosis and vertical contractions counted from videorecordings of labor in the six hours prior to delivery of the first pup ($n = 8$ dams). Based on data from [12].

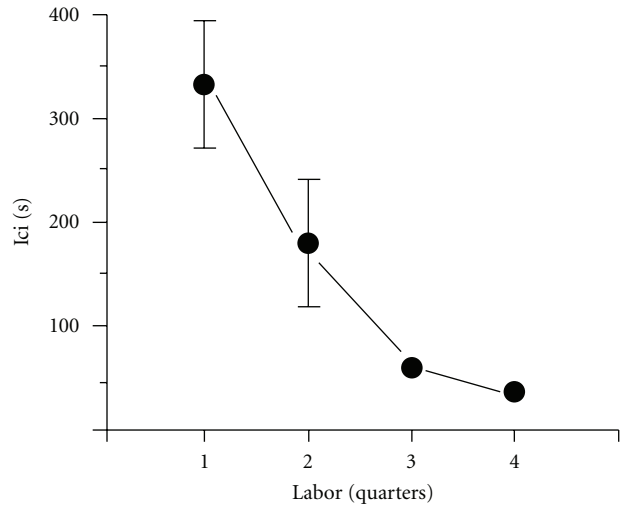

Figure 2: Intercontraction intervals (ici) during pre-birth labor recorded from rat dams ($n = 8$) from [12].

After systematically describing and analyzing the kinds and amounts of stimulation sustained by rat fetuses as they were being delivered vaginally, we endeavored to assess quantitatively some of the most prominent forms of stimulation. Among the forms of birth stimuli that we analyzed were uterine compressions, cooling and rewarming, and maternal licking. From these analyses, we created a set of procedures and tools to mimic the biological stimuli that represent the physical bases of the pups' experience of being born.

Uterine contractions, for example, were measured by surgically removing a single fetus from one of the paired uterine horns of a G18 rat installing a small balloon in its place *in utero*. The balloon was connected to a thin polyethylene tube that ran subcutaneously to the dam's back and was externalized at the nape of the neck [19]. The tubing could be connected to a pressure transducer with which we measured the forces exerted on the fetuses by the mother's behavior and by uterine contractions. The dams' contractions ranged from 2 to 30 mm Hg. By attaching an inflated balloon to a small, spring-based calibrated scale, we could apply with the balloon surface a reliable force of 15 mm Hg to a single fetal rat or to a newborn (see Figure 3). In this way, we established a protocol for simulating a vaginal birth for rat fetuses: 15, 20 sec-long compressions delivered at a rate of 1 per min, cooling (22°C), stroking with a soft brush (2 min), and rewarming (33°C).

Several of our analyses have focused on the how the birth process, beginning with the mother's labor contractions, helps organize the fetal-to-neonatal transition. Breathing and suckling are two vital behavioral adaptations of the newborn. In one set of studies, we applied our tools to study the components of birth that are important in the onset of pulmonary respiration, perhaps the most essential and immediate requirement of the newborn. The respiratory movements present *in utero* are episodic and unrelated to gas exchange [22, 23]. At birth, however, breathing becomes continuous and regulated to meet the newborn's oxygen requirements [24]. We found that compressions simulating uterine contractions were necessary for initiating breathing in late gestation rat fetuses. The effectiveness of simulated labor contractions could arise from some mechanical (nonsensory) effect of the compression, or cutaneous (sensory) effects on the offspring. In a study of gentle stroking of cesarean delivered pups (without simulated labor contractions), only 25% nonstroked pups survived for 1 hr postpartum compared to 100% stroked pups supporting a role for sensory stimulation. These observations fit well with reports of adaptive neuroendocrine changes and neurobehavioral advantages in neonates, both term and preterm, exposed to tactile and kinesthetic stimulation [25, 26].

We applied our "simulated birth" paradigm to examine more complex behavioral patterns in newborn rats in a study of how suckling becomes established [18]. Fetal rats were either exposed to labor contractions or not then cesarean delivered as described earlier, except that we manipulated postpartum ambient temperature using one of three biologically relevant temperatures. Newborns were exposed to the cool room-temperature environment (22°C) or to a warmer temperature maintained at nest (33°C) or intrauterine (36°C) temperature. After 1 hr postpartum exposure to one of the three temperature regimens, pups from all groups were placed at nest temperature then tested for nipple attachment. The 22°C condition contained the sequence of thermal exposures experienced by a vaginally born rat pup under typical thermal conditions. The treatment regime, then, was designed to represent the sequence and duration of stimulation that normally occurs prior to and immediately after vaginal birth, leading to the onset of suckling. At 2 hr postpartum, we found that 90% of vaginally delivered pups attached to a nipple (Table 1). For the cesarean newborns, both prenatal compression and postpartum temperature affected nipple attachment. The most dramatic effects of prenatal compression were seen between pups that experienced thermal conditions similar to those of normal, vaginally delivered pups (i.e., the room temperature condition), whereas thermal effects were most evident in pups exposed to atypically warm temperatures (i.e., the intrauterine temperature condition). Suckling was dramatically enhanced in compressed pups that underwent the naturalistic cooling episode.

These studies link the major postnatal milestones of pulmonary ventilation and suckling to birth experience. We sought to determine the mechanisms underlying the effectiveness of birth stimuli in facilitating the fetus-to-newborn transition. Human babies show a surge of plasma catecholamines associated with the "stress of being born," a physiological response to labor and squeezing through the birth canal [27, 28]. Vaginally delivered infants show exhibit both enhanced respiratory performance and increased alertness compared to Cesarean-delivered infants whose mothers did not undergo full labor [29–31]. Catecholamine concentrations are higher in vaginally delivered human infants as compared to Cesarean-delivered infants [32]. We analyzed plasma catecholamines at 0 to 2 hr-old following either: (a) vaginal birth, (b) cesarean section with simulated labor contractions, or (c) cesarean section without labor contractions

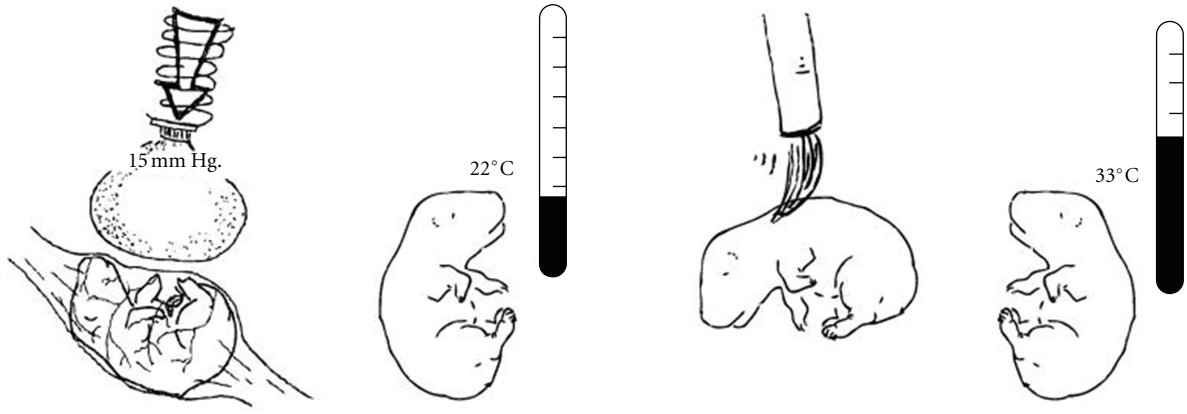

Figure 3: Four stages in a protocol for simulating a vaginal birth sequence. The drawings depict, from left to right: Simulated contractions delivered 15 times to a fetus still *in utero* but externalized from the dam's abdomen, once per minute for 20 sec each from a balloon attached to a calibrated (15 mm Hg) spring-loaded device; one hour of cooling after caesarian delivery, simulating a typical challenge faced by pups lying in the vicinity of their parturient dam still busy delivering other pups; two minutes of stroking with a soft brush, mimicking the postpartum licking by the dam; one hour of rewarming, simulating the heat received from the dam's body after being gathered and assembled in a nest with newborn littermates.

(mimicking planned cesarean delivery). Pups were exposed to the major elements of the rat's natural birth process, as we have described (i.e., umbilical cord occlusion, tactile stimulation and cooling). Only pups exposed to actual or simulated labor showed an immediate and profound rise in norepinephrine and epinephrine, to levels up to 35% greater than those of noncompressed pups. Our results, the first reported in the perinatal rat, closely parallel those reported in human studies and studies using the precocial sheep model [27, 33].

1.1. Catecholamine Release and Neonatal Adaptation to the Extrauterine Environment. Labor contractions do more than move the fetus through the birth canal. Whether by design (natural selection) or by incidental effect, contractions provide a form of stimulation that serves to facilitate two neonatal achievements: pulmonary respiration and suckling. Birth stimuli, that is, the range, levels, and patterns of stimulation that comprise the birth process, might have multiple roles in the successful transition from fetal to postnatal life [34]. Our simulated birth model incorporates actual forms and levels of sensory and physiological stimuli to which the rat is exposed during natural vaginal birth and allows us to specifically parcel out the effects of labor on postpartum functions.

The experience of labor is associated with a number of positive neonatal outcomes, including lung compliance, respiratory integrity [35–37], blood flow [38], resistance to oxidative stress [39], neonatal neurological condition [40], and complex global EEG patterns [41]. Human infants are particularly responsive to odors emanating from their mother's nipple/areola region and can identify the nipple by smell [42, 43]. Amniotic fluid and breast odors are regulators of infant sucking behavior, comfort, and distress reactions [44–46]. Learning about natural breast odors is enhanced in neonates that experience labor contractions, possibly mediated by NE [47]. Together with the results reported herein, these studies support the view that prenatal events associated with labor initiate a cascade of neural, physiological and behavioral changes that assist the neonate's successful transition to postnatal life events that assist the newborn infant's adaptation to the extrauterine world.

Table 1: Percentage of newborn pups attaching to nipples of anesthetized dams ($N = 9$ per condition). $^*P < .05$

Group	% attached
Vaginal birth (22°C)	90
Cesarean birth	
Room (22°C)	
Compressed	89*
Noncompressed	44
Nest (33°C)	
Compressed	56
Noncompressed	67
Intrauterine (36°C)	
Compressed	89
Noncompressed	78

1.2. Experiment: Simulated Birth Experience Is Sufficient to Induce Odor-Guided Nipple Attachment. We now describe an original experiment conducted in our laboratory by Abel [48] in which individual, externalized, near-term rat fetuses received a combination of the simulated birth stimuli described earlier (see Figure 3) while in the presence of the odor citral and then tested for their responses to a rat dam

with natural odors, washed of natural odors and with citral added. Specifically, while still residing in their amniotic sac and uterine horn that had been gently brought outside the dam's abdomen, each pup received a series of simulated labor contractions. Pups were next removed from the uterus, at which time there occurred a bout of tactile stimulation associated with removal of the birth membranes. Following this birth, each pup was stroked with a soft brush, mimicking the normal maternal licking and it also experienced cooling as it would after a natural birth and then rewarming as it would, had it been brought into the nest for maternal brooding. In effect, we created a simulated birth sequence. One set of pups experienced their birth in the presence of citral that was injected into the amniotic fluid prior to the intrauterine compressions and that was in the air around the pup while it was stroked and cooled and rewarmed. Alternatively, saline was used instead of an odorant for the littermate control subjects, that otherwise experienced the same birth sequence. The goal was to test the hypothesis that an arbitrary odor, paired with the experience of birth stimuli, would become a conditioned odor capable of evoking nipple attachment behavior from a newborn. The nipple attachment test was conducted with each rewarmed pup.

Our regime of stimulation was a controlled, 135 min analog of Pedersen and Blass' [16] 50-hr-long process used to induce a newborn rat's nipple attachment to novel odor. In contrast to their approach, we were able to specify and control the kinds, quantities, and timing of a specific stimulation sequence, and to observe the perinate at each stage of experimental manipulation.

We predicted that a perinatal sequence of experiences in association with an otherwise neutral olfactory cue would lead to rates of nipple attachment to that cue, similar to those of vaginally delivered newborn rat pups to the odor of amniotic fluid. If the outcome of the simulated birth experience was equivalent to a natural delivery, we would consider this a successful empirical demonstration of sufficiency. We will have demonstrated that the experience of a simulated birth, quantitatively comparable to a natural, vaginal birth, is sufficient to establish a conditioned response to an odor that is expressed as nipple attachment and the onset of sucking in an, otherwise, naïve newborn.

2. Methods

2.1. Subjects. Animal experimentation was conducted in accordance with the guidelines of the Indiana University Institutional Animal Care and Use Committee and the NRC Guide for the Care and Use of Laboratory Animals *(copyright 1996, National Academy of Science)*. One hundred twenty-six fetal rats, derived from 24, time-mated Sprague-Dawley rat dams *(Rattus norvegicus)* were used as subjects. All breeding and maintenance was conducted in the Animal Behavior Laboratory at Indiana University. The first day that sperm was detected in a vaginal lavage was recorded as the day of conception (Gestational day [G]0), with birth expected on G22. Dams were group-housed in maternity tubs under standard vivarium conditions.

2.2. Treatment of Dams. On G21, pregnant dams were briefly anesthetized with isoflurane (Aerrane, Ohmed PPD Inc., Liberty Corner, NJ). An area overlying the lumbar region was shaved and a small (3 cm) dermal incision exposed the vertebral column. To eliminate movement and sensation below the ribcage, 100% ethyl alcohol (0.1 mL) was administered via intrathecal injection between the T12 and L1 vertebrae. After confirming loss of sensation, the female was placed in a Plexiglas holding apparatus and her lower body immersed into a heated (37.5°C ± 5°C) saline bath. A midline laparotomy was performed and the dams' paired uterine horns were gently externalized into the bath.

2.3. Treatment of Fetuses and Neonates. Figure 4 depicts the prenatal and postnatal manipulations. For each dam, either citral (Sigma Chemical Co., St. Louis, 50 μL in 4 mL/L isotonic saline) or vehicle alone was injected into the amniotic fluid surrounding target fetuses. Beginning with the fetus in the second ovarian position, amniotic sacs of three-to-four adjacent fetuses were injected. A 30 ga hypodermic needle was inserted through the transparent uterine wall and into the amniotic sac near each fetus' snout.

Immediately following either citral or saline injections, compressions of 10–15 mm Hg pressure were administered to fetuses in one uterine horn using a small latex balloon. Such pressures are within the range of pressures typically experienced by rat fetuses during labor contractions [49–51]. Compressions were delivered at the rate of one, 15 sec compression per min for 15 min.

2.4. Cesarean Delivery Procedure. Upon completion of the compressions, an incision was made along the antimesometrial border of each uterine horn. Fetuses were removed individually from the uterus and delivered onto gauze pads moistened with either citral (1 mL of 4 mL citral/L isotonic saline) or isotonic saline-moistened (1 mL) gauze pads (Figure 4).

Immediately following delivery from the uterus, two cotton-tipped swabs were used to remove the birth membranes from the newborns, umbilical cords tied with surgical silk, and placentas removed. Each neonate was stroked with a soft-bristled artist's brush until respiratory activity was established (approximately 2-3 min per litter). Next, the newborns experienced temperature fluxes similar to those observed after a natural birth sequence in the laboratory [12]. They were placed onto saline-moistened gauze pads in individual glass dishes (Pyrex 80 × 40) at room temperature (22°C ± 0.5°C) for 60 min, then moved to an incubator maintained at nest temperature (33°C ± 1°C) for an additional 60 min. At 60 min postpartum, pups that had received pre- and postnatal exposure to citral were placed individually in glass dishes on citral-moistened gauze pads (1 mL citral solution/pad). These pups remained in the warm citral ambience for 5 min then transferred back to the original dishes containing saline pads in a noncitral incubator for the remainder of the second postnatal hr. Pups in both conditions were handled identically throughout the experiment except for the presence of citral. To ensure olfactory isolation, care was taken to

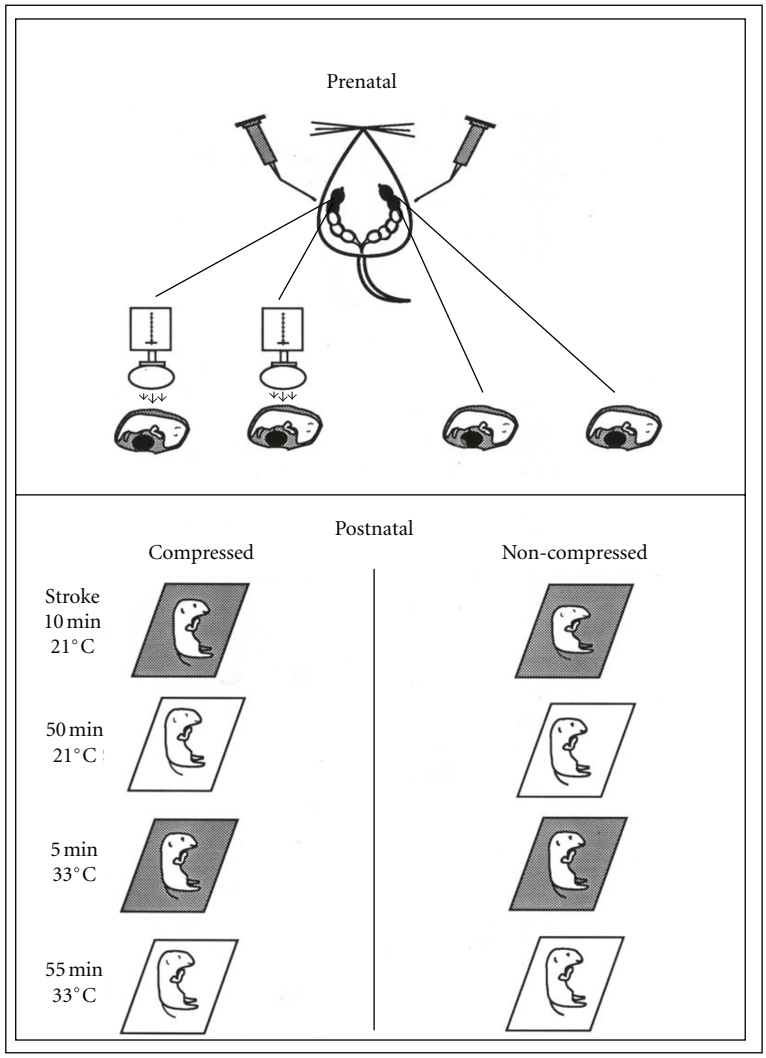

Figure 4: Sequence of perinatal manipulations prior to tests of respiration and nipple attachment. The top portion of the figure depicts injections of citral or saline into individual amniotic sacs, which was followed by compressions or no compressions (control). Pups were then delivered by caesarian section. Over the next two hours, individual pups were treated with thermal and odor regimes designed to mimic early postnatal events, shown in the lower sections of the figure. For some pups, citral was present prenatally and postnatally. Saline was used on the gauze pads of the control pups (see text). The grey and white areas around each pup depict the olfactory treatment (citral or saline control) of the moistened pads.

maintain separate citral and noncitral incubators during the postnatal exposure and testing periods.

2.5. Postnatal Respiratory Behavior. Physical stimulation in the form of compression or stroking is necessary for the establishment of respiration in newborn rat pups [19, 20]. In the present experiments, postnatal stroking was required to elicit respiratory activity in the noncompressed subjects; equivalent durations of stroking were provided to all groups. To verify that alterations in frequency of suckling onset between compressed and noncompressed newborns were not related to deficits in respiration, respiratory movements were sampled (1 min) at 3 postpartum time points: 10 min postpartum; 1 hr postpartum (while at 22°C); 2 hr postpartum (while at 33°C).

2.6. Nipple Attachment Test. Approximately 20 min prior to testing nipple attachment, a recently parturient (1-2 day postpartum) dam was anesthetized with a ketamine/xylazine mix (ip; 100 mg/mL; 0.9 mL/kg, 20 mg/mL; 0.5 mL/kg) and placed within a 33°C test incubator in the supine position. At 120 min post-delivery, each pup was gently grasped and held for up to 120 sec with its snout in contact with a nipple of the test dam [16]. Successful attachment to a nipple was verified visually and then by testing if the pup maintained oral grasp of the nipple while gently retracted from the dam.

Following the first attachment trial, the dam was moved to a second heated (33°C) incubator and citral-scented gauze pads (5 pads; 1 mL citral solution/pad) were rubbed across the ventrum, thus infusing the fur with citral odor. Note that the solution was not placed directly on nipples. The scented pads were then placed alongside the dam, further

contributing to the citral odor within the incubator. The nipple attachment test was repeated. Then, to further verify the effectiveness of perinatal exposure to citral in promoting nipple attachment to a citral-scented dam, one group of compressed but citral-naïve newborns was tested first on a citral-scented dam and then on a normal dam.

2.7. Statistical Analyses. McNemar chi-square for dependent measures was used to analyze frequency of nipple attachment. A repeated measures ANOVA was performed on respiration scores. Posthoc comparisons were made with Newman-Keuls with a cutoff of $P < 0.05$.

3. Results and Discussion

The nipple attachment test used in the present experiment reveals robust and reliable behavior in newborn rats. The leftmost histogram bar in Figure 5 shows that 90% of the vaginally-delivered newborns held before the ventrum of a natural (unwashed) anesthetized dam attached to a nipple and suckled. Note that this was the *first attachment* for each pup. Thus, this testing method enables rapid and reliable expression of the onset of postnatal ingestion, and the 90% attachment rate following vaginal birth can be used as standard against which we can evaluate the results of the simulated birth experiences.

Newborn pups that experienced a simulated vaginal birth in the presence of natural amniotic odors, including the regime of *in utero* compressions—Caesarean delivery—membrane stripping—cooling—stroking—rewarming (see Figure 3) attached to a nipple in 89% of the tests, as shown by the first hatched bar in Figure 5. The legend under that bar, Amniotic Fluid/Natural, indicates that these pups experienced unadulterated amniotic fluid odors and were with a natural, unwashed dam. Newborns that experienced the a simulated vaginal birth *absent compressions* in the presence of natural amniotic odors, attached in only about 44% of the trials, which was a significant decrement in relation to littermates treated identically but with the compressions. The contrasting result is seen in the open bar next to the hatched bar in Figure 5. Thus, the complete simulated birth sequence (including compressions), produced rates of nipple attachment in newborns that were fully comparable to those in vaginally delivered pups.

Newborn pups that experienced a simulated vaginal birth in the presence of citral in their amniotic fluid and in the atmosphere during stroking (Figure 4) attached to a nipple in 89% of the tests with a citral-scented dam, as shown by the stippled bar in Figure 5. Newborns that experienced the simulated vaginal birth *absent compressions* in the presence of natural amniotic odors, attached in only about 20% of the trials with the citral-scented dam, a significant decrement in relation to littermates treated identically but with compressions. The open bar, adjacent to the stippled bar in Figure 5, shows the contrasting outcome. Thus, the simulated birth sequence in the presence of citral, including compressions, produced rates of nipple attachment to a citral-scented dam that were fully comparable to those seen in vaginally-delivered pups and to pups that experienced the full simulated birth in the presence of amniotic odors when tested with a correspondingly natural-scented dam.

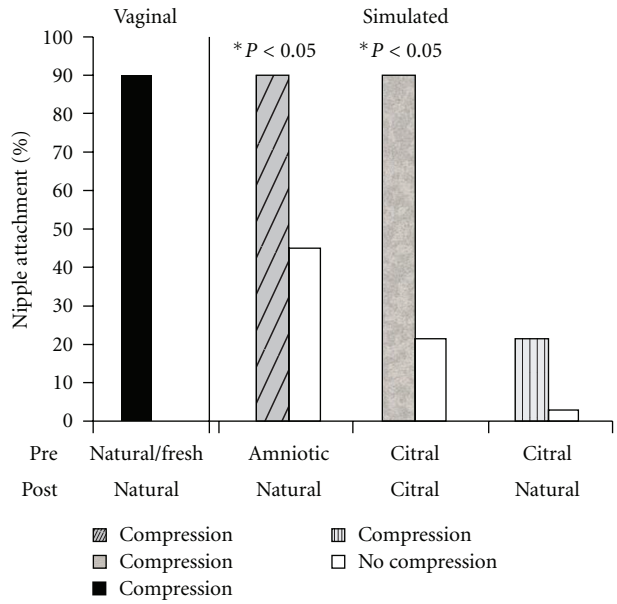

Figure 5: Percentage of pups attaching to a nipple of an anesthetized dam during a standardized suckling test. Shown below the horizontal axis are the prenatal olfactory conditions for each group and the lower row shows the odor conditions during postnatal treatments. The leftmost (black-filled) bar illustrates the baseline rate of attachment by vaginally delivered pups in these nipple attachment tests when the pups were exposed prenatally to natural amniotic odors and tested with a natural-scent dam (Vaginal group). The groups that received simulated birth experiences with different odors were also differentiated by the presence or exclusion of compressions. Within the set of Simulated birth groups, pups compressed in the presence of natural amniotic odors attached to natural-scented dams at rates comparable to the vaginally-delivered pups, but those that lacked compressions did not. If pups receiving compressions in the presence of citral (grey bar) also attached at the high rate to a citral-scented dam, but uncompressed pups in that group did not. The rightmost, striped histogram shows that pups receiving compressions in the presence of citral did not attach to natural-scented dams.

The rightmost pair of histograms show that newborns that experienced simulated birth stimuli in the presence of citral in their amniotic fluid and in the atmosphere during stroking (Figure 4) when tested with a natural scent dam (no citral during the test) attached to a nipple in only 20% and 4% of the trials, for the compressed and noncompressed subjects. Clearly, newborns that experienced birth in a citral environment were not prepared to attach to nipples on the body of a dam with only the natural scent of the species. But, we know that these newborns are capable of attaching to a nipple, as evidenced by the performance of the simulated birth group depicted by the stippled bar. For the citral-birthed pups, citral had become a necessary stimulus for the initial attachment.

Respiratory rates at the three, sampled time-points (10, 60, and 120 min) were unaffected by either citral or compression ($P > .10$). As expected, however, there was a significant increase in respiration within all groups during the final hour at the warmer temperature ($F(2,120) = 320.8$, $P < .01$).

We see at least two broad findings in the results of this experiment. The absence of *in utero* compressions of the fetus was associated with poor performance in the onset of nipple attachment. It might be tempting to conclude that compressions mimicking labor contractions are necessary for efficient initiation of nipple attachment in the newborn, but the present experiment was not designed to allow such a conclusion. Pups in the Compressed and Noncompressed groups received other tactile and thermal experiences. Although we categorized each operation as a separate form of stimulation, the perinate might be less discriminating in its responsiveness and all forms of stimulation might simply be additive and incrementally increase the level of arousal in the pup. Thus, compressions might just add to the experience of general stimulation in the pup and nipple attachment rates might reflect levels of general arousal. Even if true, such an effect would not account for the second broad finding, that odors paired with birth stimuli become conditioned stimuli for nipple attachment. The experience of a vaginal birth, real or simulated, appears to give behavioral meaning to the odors experienced in association with the birth stimuli. Schaal and colleagues have suggested that amniotic odors provide a "bridge" from the fetus' prenatal world to its postnatal environment [52], and the present results suggest that this bridge is constructed by the experiences embedded in parturition and that they result in a newborn behavior that has been rapidly assembled to follow the bridge to a nipple and the onset of suckling.

Stimulation associated with labor and delivery plays a key role in assisting the fetus' transition to postnatal life by inducing and canalizing specific behaviors, and thereby operating as a critical link in the chain of behavioral adjustments required for adaptation to the postnatal habitat. Fetal sensory experience appears to set into motion physiological processes that permit the onset of postpartum behavior and the expression of early learning.

4. Conclusions and Reflections

It makes sense, both logically and scientifically, to discard the idea that suckling is an "innate" or "instinctive" or "hard-wired" behavior in a newborn baby. Nevertheless, it is also sensible to revel in the readiness and competence of a newborn mammal to adjust immediately to severance of its umbilical connection to the uterine world and to make an oral connection to the mother's body and begin suckling. We recognized the ability of a newborn mammal to suckle by designating it as a *congenital behavior*, that is, present at birth. Clearly, suckling is an important congenital behavior worth understanding—for it is one of the primary adaptations to newborn life for all infant mammals, serving not only nutritive, immunological, and general physiological functions, but it is also a powerful component of bonding with the mother and creating a social context which supports sensory, motor, and cognitive development.

Much of what we explored in the present paper are lines of research that have been important in demystifying the kinds of basic processes that can explain the newborn's congenital abilities to orient to novel features on the mother's body surface and to initiate the complex, but vital behavior of suckling. From the findings that we reviewed, it can be concluded that the combination of sensory and motor processes that constitute successful suckling are rapidly assembled during the course of perinatal events. We focused on the roles of birth stimuli and specifically on the *experience of being born.* In the experiments we described, rat pups that did not experience the mechanical and thermal forces associated with vaginal birth failed to make the fetus-to-newborn transition. Moreover, by providing individual perinates with a simulated birth experience, it was possible to induce in them the dramatic developmental changes that serve the transition from fetus to newborn.

There is now abundant evidence that *learning* is an important component of the birth transition in rats and in humans. Thus, this perinatal learning takes place in the context of the experience of being born. It appears that the set of sensory, endocrine, and neural events that comprise the physiological transition of birth also serve as factors in the perinate's learning about the odor cues that are present *in utero* and carry over into the *ex utero* world. These are the same cues that the newborn then uses to orient to the mother's body and that stimulate nipple attachment and suckling. We are impressed by the multi-leveled functions of sensory and physiological events of birth, but much more remains to be understood about them and how they operate during parturition.

Here, we can speculate on some of the implications we see when considering the experimental findings from rats in the context of human births and the onset of suckling. As students of mammalian development, we hold an inclusive view of the basic processes comprising reproduction and development. We tend to see commonalities across mammalian species in these realms. Indeed, our past initial analyses of rat parturition (e.g., [12, 19, 20, 34, 51]) were shaped by Lagercrantz and Slotkin's [28] perspective on the "stress" of human vaginal birth. The results of our experiments with rats resemble their observations with human birth, and we have been able to take advantage of opportunities to control and manipulate the birth stimuli to gain insights into the embedded and embodied learning processes. We are particularly struck by the contrasting picture presented by prematurely-born infants who enter the postnatal world at a stage of development when their sensorimotor function is not yet prepared for suckling. While it may be beautiful and exciting to witness the eventual onset of sucking in a baby born at less than 30 weeks of age, it is sobering to contemplate the dramatically different factors and unnatural schedule of experiences that direct the prematurely-born baby to suck: a premie's early postnatal development may be supported by intravenous nutrition and then gastric intubation until the baby presents signs of "readiness" for oral feeding. Nurses, therapists and parents may then use a variety of techniques

to gently and gradually facilitate the transition to sucking and ingestion. How different it is for the baby born at term, for whom the process is short, and in many regards, even intense and abrupt. Clearly, babies can achieve successful oral ingestion via different developmental paths. We see great potential in understanding the necessary and sufficient developmental steps as precursors to improved management and guidance of early ingestion.

Other researchers have compared the development of feeding skills of term babies and prematurely born infants. Schaal and colleagues (cf., [52]), for example, have focused on the absence of pairings of chemosensory cues and nutritive intake when babies are fed by gavage. Their perspective extends to many aspects of experience that normally contribute to the integration of breathing, suckling, and swallowing. Recognition of such differences may be an important step towards identifying factors that contribute to the problems experienced by some babies and that lead to the higher incidence of feeding disorders in children born prematurely (cf., [53]).

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Review Article

Evolution and Development of Dual Ingestion Systems in Mammals: Notes on a New Thesis and Its Clinical Implications

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Traditionally, the development of oral feeding is viewed as a continuous, unitary process in which reflex-dominated sucking behavior gives rise to a more varied and volitional feeding behavior. In contrast, we consider the thesis that the infant develops two separable ingestive systems, one for suckling and one for feeding. First, we apply an evolutionary perspective, recognizing that suckling-feeding is a universal, mammalian developmental sequence. We find that in mammalian evolution, feeding systems in offspring were established prior to the evolution of lactation, and therefore suckling is a separable feature that was added to feeding. We next review an experimental literature that characterizes suckling and feeding as separable in terms of their topography, sensory controls, physiological controls, neural substrates, and experience-based development. Together, these considerations constitute a view of "dual ingestive systems." The thesis, then, is that suckling is not a simple precursor of feeding but is a complete behavior that emerges, forms, and then undergoes a dissolution that overlaps with the emergence of independent feeding. This thesis guides us to focus differently on the challenges of properly managing and facilitating oral ingestion in infants, especially those born preterm, prior to the developmental onset of suckling.

1. Introduction

The development of oral ingestion in mammalian infants proceeds in a distinct and invariant sequence: suckling from a nipple is followed by a transition to independent feeding. Conventional wisdom is that early ingestion (suckling) is dominated by reflexes and by centrally generated behavior patterns that give rise to a more complex, varied, and volitional form of ingestion (feeding). Thus, the development of oral ingestion is viewed as unitary, developmental process. This paper offers a different view of the development of oral ingestion. Put simply, our thesis is that in every mammal there are at least two, separable ingestion systems, namely, the suckling system and the feeding system. Each component of this dual ingestion system can be differentiated by its evolutionary origin, because *mammalian ingestion evolved twice*. Similarly, each component of this dual ingestion system can be differentiated developmentally because *oral feeding develops twice in mammals*. As such, proper and appropriate developmental care is aimed at protecting, supporting, and facilitating the development of two separable and specialized feeding systems—the development of suckling and the development of independent feeding.

To the parents, nurses, therapists, and physicians who care for prematurely born infants, this developmental sequence is particularly prominent, vital, and often fraught with difficulties. Indeed, infants younger than 32 weeks post-conceptional age are often incapable of orally grasping a nipple and sucking, to say nothing of coordinating sucking with breathing and swallowing [1–4]. Caregivers of infants born preterm then provide life-sustaining support while anticipating the best time to initiate oral ingestion (suckling) and then applying a variety of techniques to facilitate the further development of ingestion [5–7] so that the infant can be discharged home where continued development of feeding behavior will occur.

In the present paper, we follow the terminological rules suggested by Hall et al. [8] for discussing the processes

involved in milk transfer from mother to offspring. *Lactation* refers to the physiological state associated with milk production and secretion. *Nursing* comprises the behavioral and physiological activities that promote milk transfer to offspring. *Suckling* refers to the behavior of the young that contribute to the receipt of milk from a nipple or teat. *Sucking* consists of the oral motor movements that typically produce the intraoral pressures that help express milk from the nipple (see [8, 9] for a more complete discussion and definitions). In addition, *feeding* refers to the oral ingestion of nutritive substances other than milk, usually but not necessarily in solid or near-solid forms that typically are chewed. Finally, *ingestion* is used to encompass all forms of oral intake and includes both suckling and feeding.

We will briefly describe the *evolution* of lactation and mammalian ingestion and its relationship to suckling and feeding in the human. We will then summarize a set of *developmental* analyses from nonhuman research that compare a variety of perspectives on suckling and feeding behavior. Finally, we will identify some implications for a research agenda and for improving outcomes in children who are born preterm.

Table 1: Table of geological timescales used in this paper. The nomenclature and dates are based on those in [12, 13].

Cenozoic era	
Quarternary period	1.6–0 Myr
Tertiary period	66.4–1.6 Myr
Mesozoic era	
Cretaceous period	144–66.4 Myr
Jurassic period	208–144 Myr
Triassic period	245–208 Myr
Paleozoic era	
Permian period	286–245 Myr
Carboniferous period	360–286 Myr
Pennsylvanian (later)	
Mississipian (earlier)	
Devonian period	408–360 Myr
Silurian period	438–408 Myr
Ordovician period	505–438 Myr
Cambrian period	570–505 Myr

2. The Evolutionary Context of Lactation and Suckling Young

The vocabulary of geological timescales will be used to frame and discuss the evolution of mammals, the origins of their lactation, and the evolution of suckling offspring. We will take a rapid and abbreviated trip through geological time, sampling representative evidence that constitutes some of the fundamental understanding of mammalian origins. Within the timeline of the story, we will note the evidence pertinent to the evolution of lactation. This is uncertain territory, but we can draw liberally from a remarkable synthesis of paleontological, physiological, histological, and comparative data, recently proffered by Oftedal [10, 11] and others that provides a coherent and exciting picture of when and how lactation may have emerged to become a unique, universal, and defining feature of mammalian life.

Table 1 shows some standard divisions [12, 13] arranged chronologically from the oldest (bottom) to most recent (top). At the base of Table 1 is the Cambrian period, over 500 million years ago (mya), the period yielding the oldest known fossils of vertebrates [14]. Cambrian vertebrates were aquatic, eel-like creatures, reflected today in the jawless hagfish. These early forms proliferated and differentiated, giving rise to new types of aquatic life, such as the armoured fishes of the Silurian and Devonian periods. Reproductively, Cambrian vertebrates produced eggs that were fertilized externally. Parental care was unknown, so the offspring emerged from the egg case ready to live and feed independently. By the Carboniferous period (360 mya), there was a much greater diversity of vertebrate types. With diverse morphology came diversity in habitats and habits. Vertebrate life found its way from the aquatic medium into the air, some became amphibious, and others adopted fully terrestrial habits. Locomotion and diet evolved along with many changes in skeletal structures, appendages, and skull structure.

2.1. The Amniote Egg and the Path toward Mammals. During the Pennsylvanian, around 300 mya, there appeared a developmental innovation that proved to be a pivotal point in vertebrate evolution—the amniote egg, distinguished by additional extraembryonic membranes as well as outer layers that improved gas exchange, utilization of nutrients, and water retention [15]. Such features made it possible for the amniote-producing tetrapods to move into previously untapped habitats and accomplish successful reproduction and development. The Pennsylvanian fossil record contains a number of different amniotes that branched into separate lines, which differ by the presence and number of skull windows (fenestrae). Those lacking a skull window, or with two fenestrae, comprise the Sauropsida. One of these amniotes led to the branches comprising the turtles and another to the Diapsids that would diversify into today's squamate species, which include all lizards and snakes. The amniotes with one fenestra constitute the Synapsida. This is the line that would become the predecessor of all the mammals on Earth. Estimates are that these amniotic eggs began appearing about 310 mya.

The importance for our discussion of the proliferation of different amniote lines is that it shows that mammals did not evolve from reptiles. Rather, mammals and reptiles evolved from a common ancestry, the earliest amniotes, from which they diverged onto separate evolutionary paths more than 300 mya, denoted by the I. in Figure 1. At the demarcation of the Amniota in the figure, the divergence can be seen between Synapsida and Sauropsida. This is a noteworthy point because, as some readers will recall, early synapsids have been traditionally termed "mammal-like reptiles" ([14, 16]), a vestige of an earlier habit of calling all the early amniotes

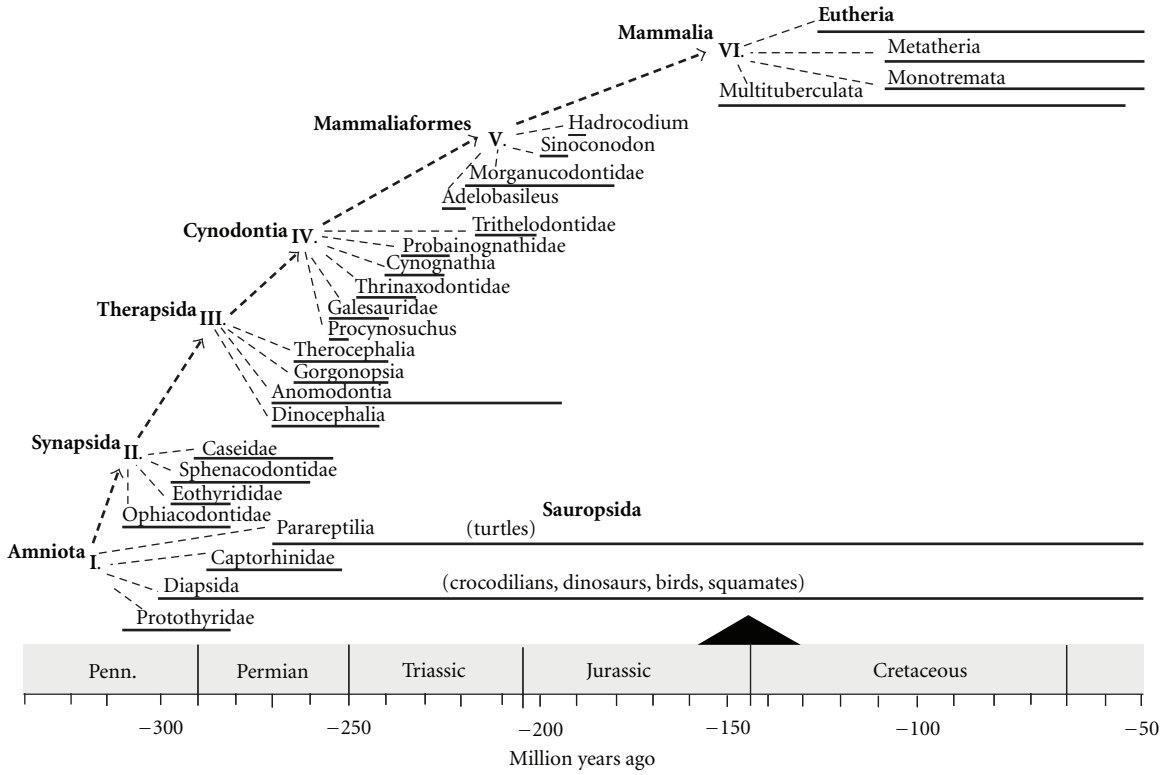

Figure 1: A simplified representation of some of the sequential, evolutionary radiations that appear from Amniota (I.) to Mammalia (II.) showing, for comparative purposes, the cladistic separation of the synapsids and saurosids as divergent subsets of Amniota. Along the dashed line, the diagram traces the trajectory from the Synapsida to the crown group, Mammalia, with select representatives of various radiating groups and species, shown by the light dashed lines. Bold horizontal lines depict the appearance and duration of each taxonomic group shown. The black triangle at the Jurassic-Cretaceous boundary denotes the end of the age of dinosaurs. The geological timescale and periods appear together at the bottom of the figure which was adapted with alterations from [10].

(both synapsid and sauropsid) "reptiles." Recognizing the evolutionary distinction between synapsid and sauropsid helps to clarify that neither mammals nor mammary glands evolved from a reptile-like ancestor with a scaly, nonglandular epidermis and calcified eggshells [11].

With the clarity of retrospection, we can see the trajectory from early synapsids to mammals. Beginning in the late Pennsylvanian period, the synapsids underwent multiple evolutionary radiations and extinctions. (Figure 1); depicts some of the new forms that appeared and disappeared along the path from the early amniotes to the Synapsida and then to the Therapsida and Cynodontia. These were eras of robust speciation for the synapsids, which became the dominant forms of animal life during the Permian and Triassic periods until the dinosaurs ascended to ruling status in the late Triassic. It was about 225 mya that mammal-like synapsid forms appeared.

Cynodonts were among the enduring groups of synapsid (Figure 2); they became abundant during the Triassic [17, 18]. Their fossils display mammal-like traits including those specifically associated with endothermy. For example, respiratory turbinals, structures associated exclusively with conservation of respiratory water and the rapid respiratory rates needed to oxygenate endothermy, are found in the nasal cavity of cynodonts [19]. Cynodont fossils also present vascularized fibrolamellar bone, suggesting rapid rates of bone growth and remodeling. In addition, there is present in Cynodont fossils a secondary hard palate that enables nasal breathing while holding prey in the mouth—which is helpful, if not necessary for a predator. Accompanying all these features are modifications of skull, jaw, and teeth that are consistent with handling the high levels of food intake needed to support elevated metabolic rates. Together, such observations lead paleontologists to date the advent of mammals as around 200 mya. Although true mammals appear to have existed during the Jurassic and Cretaceous periods, they were not widely distributed or abundant until the Tertiary, after the catastrophic ending to the age of dinosaurs.

The evolutionary path from basal synapsids to true mammals contains many transformative changes: anatomical additions, deletions, and reshaping. All of these changes reflected and contributed to modifications in physiology and function. Among the most fundamental was the gradual evolution of endothermy, which was accomplished across the mid-Permian to the Triassic. Related to the metabolic changes associated with endogenous heat production were changes in reproductive strategy, most notably the production of smaller eggs that were retained inside the mother's body. Longer periods of egg retention are associated with the eventual evolution of viviparity [10, 14, 17, 19, 20].

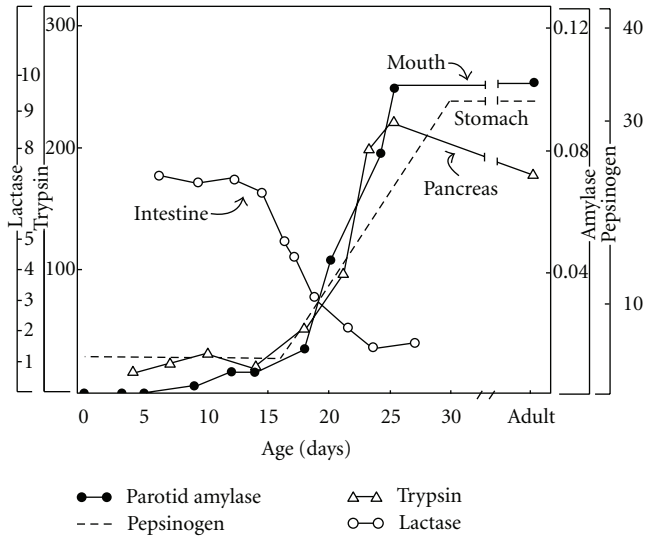

Figure 2: Developmental course of selected digestive elements of the mouth, stomach, pancreas, and intestine. Data were from [47, 50] and initially integrated in a discussion [51] that provides fuller reference information.

2.2. Origins of Lactation and Oral Ingestion within the Context of Mammalian Evolution. We have rapidly recited an evolutionary trajectory from the basal synapsids to the advent of mammals. During the 200 million years between the appearance of amniotic eggs and placental mammals, lactation began and gradually became more sophisticated. Similarly, there began the oral ingestion of maternal secretions, and this behavior gradually evolved, probably in concert with the evolving maternal lactational system, to become suckling specializations that we see in contemporary mammals. In terms of origins, however, Oftedal makes the exciting and nonintuitive assertion that lactation actually predated mammals [10]! As we will see, the origin of lactation is obscured by our knowledge of its eventual role as the nutritive foundation for mammalian newborns.

We note that originally, vertebrate evolution was based on the production of independent offspring. The calcium-based structures that define the vertebrate body-spine, skeletal appendages for walking and running, thoracic rib arrangements to enable the rapid breathing needed to support sustained exercise, skulls for the evolving brain-also produced the jaws, and teeth that enabled dietary diversification. The dentition of early ancestors tells us that they were capable feeders as growing juveniles. We can see this illustrated in the basal synapsids whose teeth were replaced continuously [17, 21], suggesting that members of every age group could feed independently [17]. Indeed, such feeding independence was an imperative, for these were offspring which experienced no parental care, representative of many highly conserved and prevalent reproductive strategies that have persisted to the present.

According to Oftedal's analysis and synthesis [10, 11], mammary gland secretions first evolved in synapsids *to provide moisture to their eggs*. That is, lactation arose as a means of protecting against dessication rather than providing nutrition. Oftedal buttresses this novel hypothesis with a thorough analysis of the physiological characteristics of "parchment shell eggs," the type most agree was produced by the synapsids and which are still produced by contemporary monotremes [11]. Oftedal's provocative suggestion would place the evolution of lactation in the Permian, some 300 mya.

Of course, the hydration of eggs through mammary secretion is not lactation as we know it today, but it is a beginning. Oftedal [10, 11] argues persuasively that lactation began well before the appearance of species we would classify as mammals. He traces the origins of lactation to reproductive strategies used in the Triassic and Jurassic, focusing on some of the mammaliaformes of that time. These were small insectivores which came in a range of sizes from about 3 g (comparable to contemporary shrews) to 25 g (mice) to 500 g (large rats). Their skeletal structures suggests that they were agile climbers (an advantage in a world dominated by dinosaurs) and probably nocturnal and elusive in their habits. These suggestions link with more specific observations supporting the idea that these early mammaliaformes were endothermic. Small endotherms require dense fur insulation and, indeed, "halos" of dense fur have been found surrounding fossils of small eutherian mammals from the early Cretaceous [22]. This is a potentially vital link to the origins of lactation, for there are a variety of proposed pathways that link endothermy to both parental behavior [20] and lactation [10].

The presence of epipubic bones in these small mammaliaformes is used as evidence for maternal care of immature offspring [10]. In addition to their role in locomotion, the sexually dimorphic epipubic bones can provide support to developing young in a marsupial pouch or to suckling young in pouchless marsupials [23]. Epipubic bones are ancient—appearing in some cynodonts and in many Mesozoic mammaliaformes [22, 24–26]. They have been lost from all extant mammals, except in monotremes and marsupials.

Oftedal projects a scenario for these small, insectivorous mammaliaformes in which the mammary patch secretions used for egg hydration became the basis of a nutrient medium for hatchlings; lactose was probably contributed by apocrine-like glands, some of which may been present in early synapsids. By the late Triassic, cynodonts were likely secreting a nutrient-rich milk, enabling a decline in egg size and an increase in altriciality of the young at hatching.

There are a host of details that support this compelling facet of evolution, but they are not vital to our discussion here. One of the central themes, however, is key: birth of immature offspring begins to develop in these early pre-mammalian forms. Dentition is again relevant, as it was earlier when we discussed the production of independent offspring. Collectively, the fossils of these small, presumably *dependent* offspring tell us that they were diphyodonts—animals with two successive sets of teeth, the first "deciduous" set and later the "permanent" set. Most mammals are diphyodonts. The demands of diphyodonty include a period of *dependent* feeding, as well as substantial acquisition of calcium, phosphorous, and other nutrients for skeletal

growth prior to independent feeding; therefore, lactation or some facultative form of nutrient provision is required. Thus, with the evolution of small, immature offspring comes the addition of a new feeding relationship and a new mode of oral ingestion.

It is noteworthy that the three taxonomic clades that comprise the existing mammals, that is, the monotremes (platypus and echidnas), metatherians (marsupials), and eutherians (placental mammals), first appear in the fossil record in the Cretaceous, but may have diverged earlier, in the Jurassic (Figure 1). It is generally accepted that oviparity (egg-laying) is ancestral to viviparity (live-bearing). It has been estimated that among reptiles, viviparity has evolved independently from a prior period of oviparity more than 100 times and never has a case of the reverse been observed [27]. Thus, it is strongly implied that monotremes use the ancestral form of parity. Evolutionarily, the progression seems to involve sequential increases in the duration of egg retention, until a condition is reached in which the egg essentially "hatches" internally, thus resulting in viviparity.

Clearly, we have drawn extensively on Oftedal's creative and scholarly efforts, for they provide a broad base of evidence for the early origins of lactation and suckling. Vorbach et al. [28] offer another perspective on the origins of lactation. They explore the idea of a development sequence in which the mammary gland evolved from simple skin glands that secreted mucuous containing a variety of antimicrobial molecules for the protection of damaged skin and only subsequently evolved nutritional components to nourish hatchlings or newborn mammals.

For our purposes, the validity of Oftedal's early hydrational origins hypothesis versus that of Vorbach et al.'s immunological protection hypothesis is not essential. In fact, the central ideas in these two innovative perspectives are not mutually exclusive and may prove to be jointly true. The essential insight, and the value of reflecting on the grand picture of mammalian evolution, is to recognize that *oral ingestion evolved at least twice*. First came independent feeding. We have considered many tens of millions of years of evolutionary history during which the many and various forms of animal life produced and reproduced offspring that hatched from eggs (fertilized externally or internally) into a world in which parental care was nonexistent and from which they had to feed independently. Thus, the sensory apparatus, motor systems, digestive capabilities, and nervous systems of all these juvenile forms were such that they were capable of immediate independent feeding. There is ample evidence of such life forms today, all descendants of ancestors common to the taxa that subsequently evolved altriciality and parental care.

Thus, brain-body systems for independent feeding is the ancestral state. Lactation and other parental secretions that can provision the young evolved subsequently. As we have seen, lactation did not evolve on its own. It was accompanied, probably facilitated by coevolutionary pressures on altriciality of offspring as well as novel adaptations in the offspring. Undoubtedly, some of the features that were or became specializations for suckling can be traced directly to adult adaptations, such as the bony secondary palate. This feature of certain synapsids, posited to have been selected as a facultative feature for predatory feeding, is an essential precondition for breathing while sucking.

Evolution, then, created and honed a constellation of specializations in increasingly altricial offspring that enabled exploitation of hydrational, nutritive, and immunological provisioning by the parents. These specializations were added to an existing system for feeding and they were added during relatively early phases of development, rather than tagged onto the end of an existing developmental sequence. In the language of heterochrony, the addition of a new feature at an early stage of development is termed "predisplacement," [29, 30] and this would be the case for the addition of suckling to a preexisting system for independent feeding. The result, then, is the evolution of two feeding systems—the ancestral feeding system and the specialized, limited, and more modern addition of a suckling system. In this way, oral feeding is not unitary and should be appreciated in terms of its evolution as having two separable heritages.

Table 2: Comparisons applied to sucking and feeding behaviors that are used to evaluate whether the development of oral ingestion is better understood as a unitary process or should be dissociated into separate developments.

Sensory controls
Motor patterns
Digestive systems
Hormones and peptides cues
Physiological regulations
Pharmacological manipulations
Brain mechanisms
Experiential mechanisms
Developmental timing
Specializations of learning

3. Suckling and Feeding as Separable Systems

To support and enrich the assertion that oral ingestion is not a unitary process, we turn to years of research, mostly from the field of developmental psychobiology. That discipline offers a broad body of well-controlled studies of the ontogeny of ingestive behavior, primarily focused on Norway rat pups. We apply that literature here, treating the rat as a decent representative of mammalian development with perhaps special applicability to the development of other generalist, omnivorous feeders-including humans. Rat development has proven value as a source of insight and understanding of human development, especially in key areas of sensory, motor, and physiological development (e.g., [31–36]). We will make the case that suckling and feeding are separable systems by systematically comparing them. Table 2 lists the kinds of comparisons that we will review. The interested reader may consult Hall and Williams' seminal discussion in which they compare suckling and feeding in rats [37].

3.1. Sensory Controls. Suckling by infant rats and other nonhuman species is dependent on the sense of smell. Teicher and Blass [38] washed the nipples and ventrum of anesthetized rat mothers with a fat soluble solvent or with water. Pups presented to the ventrum of a dam washed with the fat soluble solution did not attach to any of her 12 nipples, whereas pups presented with a water-washed control dam readily attached. Impressively, when the distillate of the wash fluids was applied to the dams' nipples, nipple attachment and suckling were reinstated, thus making it clear that the critical olfactory cue(s) had been removed and replaced and eliminating explanations such as masked odors or aversive residues. The dramatic finding that nipple attachment in the infant rat is wholly dependent on olfactory cues was further supported by the effects of anosmia on pups' suckling and weight gain [39–41]. Whereas anosmia can eliminate suckling in rats, it does not disrupt feeding on solid food. In fact, pups made anosmic at a weanling age, when they will both suckle and feed independently, will fail to attach to nipple but will readily eat [39].

3.2. Motor Patterns. Westneat and Hall [42, p. 539] asked a basic and blunt question, "Is suckling a neuromuscular precursor to chewing, or are suckling and chewing independent systems?" They used electromyogram (EMG) recordings from superficial masseter, anterior digastric, sternohyoideus, and genioglossus muscles during suckling and chewing by rat pups at six ages, ranging from Postnatal Day (PND) 6 to 21. Suckling behavior by rats consists of nipple attachment, rhythmic sucking, and the stretch response to milk letdown. The EMG patterns were distinct for each component. Chewing EMGs were present by PND 12 and are adult-like by PND 18–21. EMG patterns during nipple attachment and adult chewing were similar, but the other elements of sucking differed from chewing. Thus, at the neuromuscular level, there are discontinuities between the sucking and feeding patterns as well as at least one EMG continuity between the forms of ingestion.

We can also look into the brain and examine the central circuits that correspond to the muscles that suck and chew, again noting the rhythmic components of both sucking and adult chewing. Here the data come from guinea pig, a species born at a relatively well-formed stage of development. The infants both suckle and, within the first postnatal week, also chew solid food. The rhythmic patterns for guinea pig oral ingestion originate from central pattern generators in the frontal cortex of the brain [43, 44]. Neurons from this cortical area project to an oral rhythm generator in medulla oblongata [45]. It was shown that there exist at least two separate pattern generators, one for sucking and one for chewing; during the transition from suckling to chewing, there is a corresponding structural and functional transition of active neurons [46].

3.3. Digestive Systems. It is possible to dissociate the suckling and feeding systems in the developmental profiles of some of the digestive enzymes that comprise the postingestion physiology of the infant and weanling. The open circles in Figure 2 illustrate levels of intestinal lactase in the suckling rat, which are high throughout the first two postnatal weeks (when the rat is an obligate suckler) and then begin a precipitous decline during weaning [47] which, in this species, lasts until about PND 30 [33, 48, 49]. In contrast, the figure's triangles, broken line, and filled circles show levels of enzymes that help digest solid foods at different levels of the oro-gastrointestinal system—these levels rise together just as lactase is decreasing (cf., [50]) The coordinated changes in digestive enzymes in Figure 2 beautifully illustrate the developmental dissociation of the suckling and feeding systems [51].

3.4. Separable, Specific Physiological Controls of Suckling and Feeding. There are a host of endogenous hormones and peptides that modulate food intake in weanling age or older rats, but do not exert similar effects on the suckling animals. For instance, exogenous insulin is known to increase food intake and weight gain in adults, but it did not have similar effects in suckling pups [52]. Likewise, a 7-day treatment regime of ghrelin was found to increase body weight of 7-week-old rats, but the same regime applied to suckling pups did not change their weights [53]. These intriguing findings alone would be suspect because there may not have been adequate experimental measures to ensure that the maternal milk supply was sufficiently abundant to allow pups to increase their intake and accumulate additional body mass. It was also shown, however, that intraperitoneal or intraventricular administration of leptin (a putative satiety factor) decreases food intake in 28-day-old pups but does not affect suckling intake [53]. Similarly, cholecystokinin inhibits food intake in adults but does not alter suckling in 5- or 10-day-old pups [54].

3.5. General Physiological Controls of Suckling and Feeding Are Different. In a particularly broad and inventive study, Hall and Rosenblatt [55] equipped rat pups of various ages with an oral cannula through which they could infuse controlled amounts of milk, thus by-passing the limits of maternal milk supply. Cannulated pups attach to and suckle from the nipples of anesthetized dams, so this experimental preparation is highly versatile: the experimenter can provide measured amounts of milk directly into the infant's mouth even if the pup is attached to a nipple. A small amount of milk infused into the mouth of a pup attached to a nipple elicits the age-typical behavioral responses to a natural letdown. Pups readily ingested and reacted as though the dam had a milk letdown. Using this preparation, it was shown that infant rats PND 10 and younger do not inhibit intake when milk is delivered while pups are on-nipple. This is not an artifact of the cannulation preparation, however, because by PND 15, pups show satiety responses to the same type of milk transfer.

Friedman [56] concluded that under natural conditions with an awake, active lactating dam, it is maternal milk supply that limits the pups' intake. Satiety mechanisms in the young suckling pup are not involved. Preloads of milk or other forms of calories did not reduce milk intake by 10-day-old pups. Interestingly, Friedman [56] reported that preloading the pups with water did reduce their intake which

pointed his investigation toward hydrational cues, rather than nutritive controls of early suckling. He tested two types of hydrational cues, using osmotic and volemic manipulations. Rat pups at PND 10 did not alter their suckling to an osmotic challenge, but they did increase their milk intake in response to hypovolemia induced by a subcutaneous injection of polyethylene glycol, a hyperoncotic colloid.

3.6. Pharmacological Dissociations of Suckling and Feeding. Our thesis is further buttressed by contrasting results on suckling and feeding obtained with pharmacological manipulations. Amphetamine is widely recognized for its anorexigenic effects. Adult rats and humans show dose-related decreases in hunger and food intake to amphetamine. In rat pups, however, amphetamine does not decrease intake [52, 57]. Suckling rat pups given amphetamine and presented with an anesthetized dam actually attached to nipples more readily and suckled more vigorously than did controls [37, 57]. Milk intake was not part of this study because the anesthetized dam did not deliver milk, but it was striking to see a drug that dramatically diminishes an adult's interest in food actually augment the appetitive elements of suckling pups.

There is an additional series of studies that provides data suggesting differential controls on suckling and feeding by the neurotransmitter serotonin (5-hydroxytryptamine, or 5-HT). Drugs that block 5-HT receptors, such as methysergide, stimulate weanling age pups to suckle. That is, they increase sucking in pups that are developmentally disinclined to attach to nipples and suck. An exciting observation in relation to our interest in dissociation of dual ingestion systems is that 5-HT antagonists cause weanling pups to prefer to suckle rather than to eat food [58]. Furthermore, the more age-typical preference for feeding can be reinstated by treatment with a 5-HT agonist [59].

3.7. Incidental Observations Implying Separate Central Mechanisms for Suckling and Feeding. There are remarkably few data on the development of brain mechanisms for suckling and feeding. Historically, much attention was directed at hypothalamic control of hunger and satiety and the involvement of ascending pathways in and around the medial forebrain bundle, but such studies have largely yielded to investigations of neurotransmitters and systemic factors as noted earlier in our review. Electrolytic lesions in a variety of ingestion-related brain regions have generally had similar-appearing disruptive effects on both sucking and feeding, but such work has not been pursued developmentally in great detail.

Other forays into questions of central mechanisms for suckling versus feeding are similarly incomplete but intriguing. Bignall and Schramm [60] reported a developmental study of ingestion by "mesencephalic animals," kittens decerebrated within one week of birth and followed up to two months of age. They reported that the abolishment of suckling behavior was the only discernible immediate deficit and that the ingestion of solid foods and lapping of milk developed at weaning age. There are many limitations to their study, including the small sample size ($n = 5$) and the fact that the surgical transection was complete in only two of the animals. Nevertheless, they reported that suckling was permanently abolished in all the animals, but by maintaining them on gavage feeding to weaning age, feeding behavior emerged on schedule.

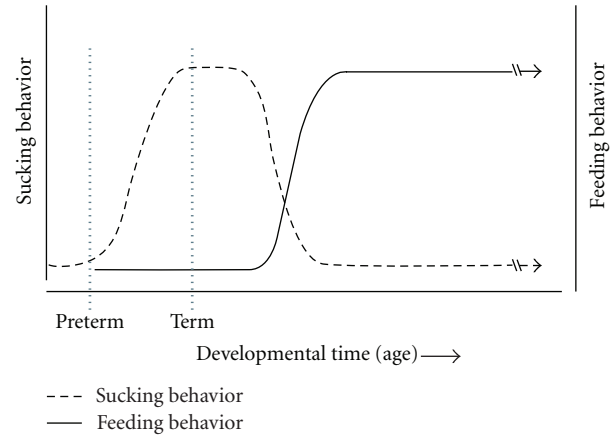

Figure 3: An idealized depiction of the coordinate development of suckling and feeding behavior which, together, comprise the development of oral ingestion. Note that the horizontal axis depicts an unspecified range of "developmental time" with vertical markers exemplifying a preterm birth and a term birth. The purpose of noting these events is to illustrate that the suckling system may not be functionally ready for the preterm infant, whereas it is prepared to be engaged at term.

3.8. The Separate Roles of Experience in the Maintenance of Suckling and Emergence of Feeding. Figure 3 is an idealized depiction of the coordinate development of suckling and feeding. The open circles depict the initial presence of suckling, its maintenance through infancy, and then its gradual decline and disappearance. The closed circles depict the gradual emergence and establishment of feeding. Typically, the two processes are synchronized (as depicted in the figure), so much so that many reasonable and thoughtful observers see the synchrony simply as the continuous development of a single system of oral ingestion. In this context, then, it was stunning when Pfister et al. [61] raised rats among litters of pups that were periodically replaced so that the subject rat was always living with suckling age pups and the mother. These subjects weaned onto solid food, but continued (even into sexual maturity) to suckle along with their preweanling counterparts! This dramatic (and bizarre) experiment tells us at least two things pertinent to the present thesis. First, the developmental dissociation of suckling dissolution from the emergence of feeding provides an important demonstration that suckling does not become feeding; the odd social context provided by these experimenters kept suckling behavior intact while the pups' feeding system separately emerged and matured. Second, it is possible to manipulate separately the ontogeny of suckling and the ontogeny of feeding, adding another type of evidence that the systems are separable.

The independence of suckling and feeding systems was echoed in a different type of experiment, the results of which strongly suggest that feeding in rats can develop without

prior experience or practice of suckling behavior. Hall [62] devised a preparation whereby a cannula is installed in the stomach of a newborn rat so that it can be raised alone, typically in a container that is kept in a warm, moist environment where a milk formula is infused directly into the stomach and experimenters periodically stimulate urination and defecation. This "pup-in-a-cup" technique has been used for a variety of preliminary investigations including questions about of the role of suckling experience as a precursor to feeding. Pups raised in isolation from the mother and fed via the gastric cannula PND 18 were then tested for their ability to feed. (Cannulation was performed between 12 and 48 hrs after birth.) Remarkably, when these suckling-deprived animals were given access to solid food on PND 19, the latency to begin eating was identical to normally reared pups. Moreover, the suckling-deprived pups performed well and even equivalently to suckled pups with compensatory feeding after 24 hr food deprivation, with compensatory drinking after water deprivation, in response to dietary adulteration, and they demonstrated equivalent food motivation by learning to press a bar for food and water as rapidly as did normal rats [62].

In contrast to the feeding system's apparent independence from prior suckling experience, the suckling system appears to depend on stimulation and experience. After as little as two days of the artificial rearing regime, including the suspension of suckling experience, pups no longer display the ability or willingness to suckle [63]. In this way, suckling and feeding are further dissociated by vulnerability to disruption. The psychobiology literature contains many examples and much knowledge of how both suckling and feeding are experience-sensitive and also how the two systems interact. Here we chose to emphasize findings that elucidate the ways in which the systems are separable, for these aspects are relatively novel and can lead us to new approaches to solve old problems.

3.9. Learning Mechanisms That Separate Suckling and Feeding. One of the most rapid and enduring associations that rat pups can learn are "conditioned taste aversions." Such learning occurs when a distinct and novel taste is followed by a treatment that causes nausea or gastrointestinal malaise, typically induced by a dose of a nonlethal toxin such as lithium chloride (LiCl). In one part of a series of experiments [51, 64, 65], rat pups were equipped with the type of oral cannula described earlier [55] that enabled precise amounts of flavored milk to be delivered by an experimenter. When milk containing a novel flavor was pulsed into a pup's mouth and this flavor experience was paired with LiCl-induced illness, the pup would subsequently avoid ingesting a food (powdered rat chow) containing the illness-associated flavor. The association was learned in one trial and the learning was strong and enduring. Remarkably, if the pup was attached to a dam's nipple when the same flavored milk was delivered to the same site of the tongue in the same amount, on the same schedule, and paired with LiCl-induced illness, the pup did not react in any way that indicated its memory of a taste aversion. A variety of control studies established that the key variable was whether the pup was in a suckling context. Like the painful shock study described earlier, the suckling context redefines the pup's learning contingencies and negative associations are not formed. This type of surprising finding has been discussed in a variety of ways [51, 66, 67] but for present purposes, it is a view of a learning mechanism that is functionally defined by whether it occurs in the context of suckling or of feeding.

3.10. Independent Ingestion by Neonatal Rat. A neonatal rat pup, at PND 3, deprived of nutrition for nearly a day and placed alone in a warm, humid chamber with a puddle of milk or wet mash on the floor will, under such conditions, orally ingest milk from the puddle. It is argued that this remarkable phenomenon is a case of regulated, independent ingestion because the behavior ceases when the pup ingests about 5% of its body weight; impressively, the amount the pup ingests is diminished by a preload of milk [68]. Recall that rat pups 10 days old and younger are demonstrably nonregulatory when they are tested on nipple. A popular interpretation of independent ingestion by an isolated 3-day-old infant rat is that it demonstrates that there exists a system for independent feeding in the neonatal brain that is normally inaccessible, further evidence of a dual ingestive system in a developing mammal.

4. Do Human Infants Possess Dual Ingestion Systems?

Treating oral ingestion as an aspect of behavioral biology makes it obligatory to consider its the evolutionary and developmental aspects. Within a biological framework, we expect the ontogeny of oral ingestion in humans to be consistent with that of other mammalian species, and that human infants also possess "dual ingestive systems."

The dual ingestive system thesis is novel, particularly in clinical domains, so it is understandable that there are few data such as those known for rodent development. Nevertheless, it is possible to note several lines of evidence that are consistent with our assertion that dual ingestive systems are a conserved feature of mammalian development that pertains to all mammalian species, including humans. Human infants, like the offspring of every mammal, begin postnatal life as monophagous organisms (those that ingest only one substance), deriving all nutrition, water, and electrolytes from a single source—mother's milk. Suckling is the singular mode of ingestion while in the developmental phase of monophagy. As in other newborn mammals, suckling is the obligatory, initial phase of ingestion for humans.

Feeding, the ingestion of nonmilk foods including solids and boluses of soft substances, typically has a gradual onset after suckling is established. In human offspring, as in most mammalian young, suckling continues to be displayed even after the onset of feeding. In fact, the developmental duration of overlap of the two modes of ingestion can be considerable, depending on a variety of contextual variables, including food availability and cultural traditions. Thus, human infants may suckle exclusively for six months and then both suckle and feed for another six months or more. Different

substances from different sources are taken with each mode of ingestion. Human suckling and human feeding behaviors are distinguishable in terms of their topographies. A host of morphological changes and muscular developments underlie age-related modifications of the tongue, and oral-pharyngeal complex helps differentiate the movements that comprise sucking and chewing in human infants (see [69] for a useful review). Similarly, the neural systems that mediate sucking (and coordinate breathing and swallowing) are separable from many of those that mediate feeding (see [69]). In such ways, as we have seen in analyses of nonhuman ingestive development, suckling does not transform into feeding; both modes of ingestion are expressed separately within the same developmental phases. That the absolute duration during which the two behaviors exist in an infant can vary greatly also suggests that feeding develops independently of suckling in humans.

Understandably, experimental studies involving some of the more dramatic manipulations imposed on laboratory species are not conducted with humans. Importantly, however, Pickler et al. [2] provide nonexperimental evidence that achieving competence with nipple feeding depends on the amount and timing of prior experience. Such observations connect importantly to our general knowledge that suckling is a complete, adaptive, integrated behavior with its own functional integrity that is shaped by experience and context.

Although we emphasize that suckling and feeding are separable along many dimensions, the two behaviors are *not separate*. The prevalence of feeding disorders among children who sustained disrupted suckling experiences [70] is an important reminder that we must pursue deeper understanding of the connections between suckling and feeding. Menella and Beauchamp's studies (e.g., [71–73]) showing that flavor experiences during suckling or even in utero can affect feeding preferences in later life indicate that there are experiential lines of continuity between the two ingestive systems.

5. How Does the Thesis of Dual Ingestion Systems Relate to the Development of Oral Feeding in the NICU?

Lactation and suckling are unique to mammals and universal among them, thus squarely placing this aspect of human reproduction and development in a broad biological context and directing our attention to evolutionary history, for this is the source of insights into comparative questions. When we examine the evolution of lactation and oral ingestion we discover beautiful continuities, as we should with any feature shared by diverse species with common ancestors.

The evolutionary story tells us that infant suckling is a "new invention" that followed the advent of live birth and immature offspring. Our evolutionary ancestry is from externally delivered eggs which hatched relatively well-formed offspring capable of independent ingestion. We saw that suckling was subsequently "invented" and therefore added to the pre-existing systems for independent, oral ingestion. When we examined the development of oral ingestion in a living relative of ours—a laboratory rodent—we discovered a swath of evidence for two ingestive systems, one for sucking and one for feeding [37]. They are separable, though the two ingestive systems are not entirely separate.

The perspective on the evolution and development of oral ingestion, as outlined here, has value and validity for practitioners and researchers concerned with oral ingestion in human infants. The perspective we have introduced can increase and sharpen our awareness of the developmental structure of oral ingestion [1–3, 7]. This alone can help define areas where knowledge is needed and help prioritize what knowledge we should apply. As such, we are better able to treat suckling as a complete behavior, not as practice for a subsequent behavior or as a precursor to feeding, but as a complex, ontogenetically complete behavior with an adaptive integrity to be attained and then followed by developmental dissolution. With the adoption of such a view, a new set of research questions can be articulated and take on a new importance. When suckling is seen as a developmental goal, the *precursors of suckling* become germane, especially to clinicians who work with premature infants whose suckling is undeveloped.

For example, swallowing might now be viewed as an important behavioral precursor to the onset and development of suckling. We know that fetuses swallow and that the behavior serves a variety of immediate and prospective functions [74]. How often, when, and how much do fetuses swallow? Does the premie swallow similarly? It seems likely that an infant, prematurely thrust into an environment where nutrients are delivered to its stomach via nasogastric tube and pulmonary breathing is not only required but is assisted with forms of pressurized airflow, will adopt patterns of swallowing different than that of a same-aged fetus. We would probably serve the infant well to understand this in greater detail.

Within the new framework, chemical cues (tastes and smells) that contribute to state, activity, orientation, arousal, and calming [75–77] and that can initiate digestive processes now have magnified potential. Through the lens of the "dual ingestive systems," we can see with greater clarity their possible importance. We are compelled to ask if there is important information in knowledge of the tastes and odors of amniotic fluid and vernix and glandular secretions of mothers and fathers. Can we use such odors to augment state regulation in the infant or to stimulate ingestion? Can we better see the natural contingencies for learning when a mother brings her baby to the breast, stimulating its senses and coaxing from it a series of new acts that are rewarded with external and internal sensations? In the context of suckling as a complete developmental system, it is possible to envision how odors might be used in the NICU to recreate some of the key contingencies and associations and facilitate the preterm infant's developmental course.

The baby's experiences are particularly significant in this framework. Experience and action are vital components in modern developmental analyses [78–81]. Our thesis provides a framework that can help order and organize experience into elements that can separately contribute to suckling and later to feeding. One kind of experience may have similar

or different effects on either or both of the two kinds of ingestion. The experimental literature can show how to recognize and to differentiate among such kinds of effects.

These are but a small sample of some of the practical implications and promises for improved practice that could evolve from the thesis we have introduced in this paper. There is need for further research, so that we might better help infants develop suckling as part of an integrated array of adaptive competencies that will enable discharge from NICU to home [3, 4, 6, 7]. As with any new formulation, it is certain that important qualifications and unforeseen implications await us. But only by adopting and applying these ideas and pursuing with research their value and utility will we be able to make the most of them and understand the extent to which and how clinical practice and, most importantly, the outcomes on babies and their families, can be improved.

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Clinical Study

Frequency Modulation and Spatiotemporal Stability of the sCPG in Preterm Infants with RDS

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The nonnutritive suck (NNS) is an observable and accessible motor behavior which is often used to make inference about brain development and pre-feeding skill in preterm and term infants. The purpose of this study was to model NNS burst compression pressure dynamics in the frequency and time domain among two groups of preterm infants, including those with respiratory distress syndrome (RDS, $N = 15$) and 17 healthy controls. Digitized samples of NNS compression pressure waveforms recorded at a 1-week interval were collected 15 minutes prior to a scheduled feed. Regression analysis and ANOVA revealed that healthy preterm infants produced longer NNS bursts and the mean burst initiation cycle frequencies were higher when compared to the RDS group. Moreover, the initial 5 cycles of the NNS burst manifest a frequency modulated (FM) segment which is a significant feature of the suck central pattern generator (sCPG), and differentially expressed in healthy and RDS infants. The NNS burst structure revealed significantly lower spatiotemporal index values for control versus RDS preterm infants during FM, and provides additional information on the microstructure of the sCPG which may be used to gauge the developmental status and progression of oromotor control systems among these fragile infants.

1. Introduction

1.1. Suck Central Pattern Generator. The mammalian suck is the earliest-appearing somatic motor rhythm and is primarily controlled by a neural network known as the suck central pattern generator (sCPG). Suck appears *in utero* between 15 and 18 of weeks gestational age (GA) and the nonnutritive suck (NNS) is remarkably stable and well patterned by 32 weeks postmenstrual age (PMA) among healthy preterm infants [1]. The presence of a coordinated NNS is a good index of sucking skills, but not necessarily of an infant's readiness to orally feed [2]. Coordinated nutritive suck-swallow-breathe (bottle feeding) is usually not attained until 34 weeks PMA [2–4].

The sCPG consists of bilateral, linked internuncial circuits within the brainstem pontine and medullary reticular formation [5–7]. Based on animal models, the minimal circuitries for ororhythmic activity reside between the trigeminal motor nucleus and the facial nucleus in the brainstem [7] and are situated to function as premotor inputs to lower motor neurons. The sCPG is modulated by multiple inputs, including descending pathways from sensorimotor cortex and reciprocal connections with the cerebellum [8, 9], which serve to modulate ororhythmic activity. Thus, it is important to assist human infants to regulate their behavioral "state" through careful posturing and orientation during clinical testing as this will affect the nature of descending inputs to the sCPG. The sCPG can also be modified by sensory input arising from oral mechanoreceptors that encode the consequences of oral movements and external stimulation (e.g., breast, pacifier or bottle nipple, and touch) along central pathways of the trigeminal system. Suck entrainment has been demonstrated in term infants through 6 months of age using a patterned orocutaneous stimulus delivered to perioral and intraoral tissues [10]. Entrainment is defined as the phase locking of centrally generated suck motor patterns to an applied external stimulus, and represents a powerful method of achieving neural synchrony among sensorimotor

pathways. Therefore, it is not surprising that stimulation of the lips and tongue is common method used to evoke sucking behaviors [11–13].

A precursor ororhythmic sensorimotor behavior involving a reduced set of orofacial muscle systems, nonnutritive suck (NNS) is typically observed in preterm infants between 28 and 33 of weeks gestation. The NNS is defined as a repetitive mouthing activity on a pacifier or a nipple without expelling any liquid stimulus [14, 15] and is thought to be regulated in part by a bilateral brainstem neural circuit known as the suck central pattern generator (sCPG) [5, 7, 16]. Wolff [15] described the NNS as changing across segments such that the first and second segments were faster than the third. Animal models suggest that the sCPG is sensitive to sensory input, modified by experience, and modulated by descending inputs from somatosensory cortex and reciprocal connections with the cerebellum. Sensitivity to punctate, low-amplitude high-velocity orocutaneous input to accelerate the development of NNS has recently been demonstrated in human preterm [17–19] and term infants [10].

Feeding readiness and inference to neural integrity is often evaluated by an infant's display of NNS and oromotor patterning [2, 20, 21]. The maturation and coordination of the NNS precedes the suck-swallow-breathe pattern associated with the slower 1 Hz pattern characteristic of the nutritive suck [22–24].

Suck is a precocial ororhythmic motor behavior in humans and is integral to competent oral feeds. However, premature infants often demonstrate oromotor dyscoordination and are unable to suck and feed orally [25, 26]. This represents a frequent and serious challenge both to the neonatal intensive care unit (NICU) survivors and the physician-provider-parent teams. The potential causes for delayed or impaired suck development are numerous and may result from neurologic insult to the developing brain, feeding intolerance, post-surgical recovery, diabetes, drug exposure, or as a result of ventilator interventions for delayed lung development or lung disease which interferes with ororhythmic pattern formation.

The interval of inactivity between NNS compression bursts is known as the "pause" period. Characteristics of the NNS can be described in terms of *coarse* and *fine* structure. The coarse structure of the NNS usually refers to a simple accounting of the number of suck bursts and pauses, respective duration, and the number and amplitude of suck compression cycles within each burst. The fine structure of the NNS defines within-burst characteristics such as the period and amplitude of each burst cycle [24] or a statistical measure of NNS burst pattern formation known as spatiotemporal stability [18, 19]. Precise measures of the coarse and fine structure of the NNS provide useful information correlated to developmental status and progression of oromotor control systems among these fragile infants [17, 18, 20, 27, 28].

The aim of the present study was to further explore the fine structure of the NNS burst using frequency and time domain measures, and exponential regression methods to capture the modulation inherent to NNS burst structure in health and disease. Such a model is likely to provide a new utility for comparison of oromotor skills across preterm groups varying in the degree of sensory deprivation/motor restriction due to O_2 supplementation therapy and can be used to monitor change in sCPG pattern formation due to maturation and intervention. In the present paper, particular attention is given to the dynamics of NNS burst initiation and frequency modulation of suck compression cycles among RDS and healthy control preterm populations. We expect to find a relation between the NNS pattern formation and RDS severity which will lead to the formulation of a mathematical and statistical model of NNS burst cycle dynamics.

Table 1

Variable	Control ($N = 17$)	RDS ($N = 15$)
SEX (male : female)	8 : 9	8 : 7
Birth GA (wks)	31.5 (1.4)	29.6 (1.3)
Birth weight (gms)	1518.7 (318.6)	1317.0 (480.8)
PMA (wks)		
Session 1	33.56 (1.7)	34.69 (1.9)
Session 2	34.66 (1.6)	35.41 (1.8)
Mean	**34.11 (1.7)**	**35.05 (1.9)**
% Oral feed		
Session 1	13.2 (4.0)	1.73 (6.4)
Session 2	34.47 (9.5)	6.00 (10.1)
Mean	**22.46 (6.8)**	**3.87 (8.6)**
O_2 history (days)		
VENT	0.00 (0.0)	5.73 (8.1)
CPAP	0.71 (1.1)	8.40 (8.6)
Cannula	0.65 (1.3)	20.07 (18.9)
Total	**1.35 (1.7)**	**34.20 (28.6)**

2. Methods

2.1. Participants. Participants included 32 preterm infants (16 F, 16 M) admitted and receiving care in the NICUs of Stormont-Vail HealthCare (Topeka, KS, USA) and the University of Kansas Medical Center (Kansas City, KS, USA). The mean gestational age (GA) was 30.61 weeks and the mean birth weight was 1424.15 grams. These infants were distributed among two groups including healthy control and RDS, according to their oxygen and percent daily oral feeding histories. At 34.11 weeks PMA, the control infants ($n = 17$) averaged less than a day and a half of oxygen therapy, no ventilation was required, and feeding 22.46% orally. Infants in the RDS group ($n = 15$) had an average of 34.2 days of oxygen therapy and demonstrated marked deficits in oral feeding, 3.87% PO (Table 1). All infants had been extubated for at least 5 days at the time of testing. Infants were consented with the additional inclusion criteria: head circumference within 10–90th percentile of mean for PMA, neurological examination showing no anomalies for PMA (response to light, sound, and spontaneous movements of all extremities), and with stable vital signs (heart rate, blood pressure, age appropriate respiratory rate, and oxygen saturation > 92 SpO_2) to allow for NNS. Exclusion criteria were intracranial hemorrhage, hypoxia-ischemia encephalopathy,

periventricular leukomalacia (PVL), neonatal seizures and culture positive sepsis or meningitis at time of testing, chromosomal anomalies, or craniofacial malformation.

2.2. Data Collection. Digital recording of NNS compression pressure waveforms was initiated after 32 weeks PMA and continued on a weekly basis until the infant was discharged or transferred from the hospital. Data considered in the present paper is based on two digitized samples, sampled at a 1-week interval of NNS compression pressure waveforms collected 15 minutes prior to a scheduled feed. Infants remained connected to their pulse-oximetry monitors during testing. The infant was placed in a developmentally supportive position, including head support, arms and hands swaddled at midline, background lighting dimmed, and facing the examiner to promote eye contact. The infant's pacifier was placed on a pressure-instrumented acetyl receiver specially designed to accommodate the Philips AVENT Soothie silicone pacifier. This configuration allowed real time sampling and analysis of the NNS using NeoSuck RT, a software program coded in our laboratory using C^{++}. A 48″ Luer pressure line coupled the pacifier-receiver handpiece to a calibrated Honeywell 5 psi pressure transducer. Transducer output was conditioned by a DC-coupled bridge amplifier (BioCom 215, Butterworth low pass @ 50 Hz). The infant-generated analog pacifier compression pressure signal was sampled in real time at 3 kHz (16-bits, National Instruments PCI-6062E).

Sampling of NNS behavior was initiated when the infant achieved an optimal behavioral state, that is, drowsy to active alert (state 3, 4, or 5 as described by the Naturalistic Observation of Newborn Behavior, Newborn Individualized Developmental Care and Assessment Program; NIDCAP) [29]. Three minutes of NNS behavior was digitized for each infant per session, with the most productive 2-minute epoch based on NNS cycle count subjected to formal quantitative and statistical analysis.

2.3. NNS Signal Analysis

NNS Compression Waveform Discrimination. Nonnutritive compression suck cycle periods were obtained from the digitized NNS pressure waveform record obtained using a waveform discrimination and pressure threshold detection algorithm coded in the NeoSuck RT software program which automatically indexes pressure peaks for signals which exceed a preset 2 cm H_2O pressure threshold. Operationally defined, an NNS burst in this context is defined as 2 or more NNS compression cycles having cycle periods of 1000 ms or less. Identification of the time-amplitude intercepts for individual pressure peaks is achieved by calculation of the first derivative of the pressure signal. A set of algorithms available in MatLAB are coded to automatically segment and index individual NNS compression cycles. The first derivative of the NNS pressure waveform is NNS pressure velocity. Pressure velocity "zero crossings" corresponds to the reversal in pressure trajectory associated with "peaks." These peak indexes tallied as time-amplitude intercepts in the digitized NNS pressure record are coregistered with a pressure "history" or hysteresis function to identify major peaks from minor fluctuations in pressure slopes. This algorithm permits objective identification of NNS burst activity as distinct from nonorganized mouthing compressions. The resultant time period is converted to frequency using the formula $F = 1/T$; where F equals frequency (Hertz or Hz). This measure represents the instantaneous cycle rate between two consecutive pacifier nipple compression pressure peaks, and T is the time period (seconds) between two consecutive compression pressure peaks (Figure 1(b)).

2.4. NNS Spatiotemporal Index. The physiological approach to the assessment and habilitation of suck in the NICU includes a functional assessment of the integrity of the neural circuitry driving the sCPG through an analysis of suck pattern structure and stability [18]. Coordinated NNS that is minimally variable from burst-to-burst indicates motor system integrity and is an important foundation for coordination with other emergent behaviors, such as swallow and respiration. A highly promising digital signal processing technique known as the NonNutritive Suck Spatiotemporal Index (NNS STI) has been developed in our laboratory to quantify the emergence of stable NNS in preterm infants. The mathematical tenets underlying this computational technique have been used successfully to assess kinematic variability and pattern formation in limb [30, 31] and speech [32, 33] motor subsystems. The NNS STI provides the clinician with a single numerical value, calculated from the cumulative sum of the standard deviations on a set of five amplitude- and time-normalized suck pressure burst waveforms. The net statistic NNS STI represents the stability of the infant's oromotor sequence. For example, an NNS STI equal to 85 represents a highly variable NNS burst structure from one production to the next, whereas an NNS STI equal to 30 represents a relatively invariant, stable pattern of suck burst output. The NNS-STI is designed to quantify the infant's suck over a selected burst pattern epoch, thereby providing NICU clinicians with a summative index or "*gestalt*" of oromotor pattern formation and stability. Obtaining a two-minute sample of NNS behavior daily in the NICU with a physiological data acquisition microprocessor at cribside is sufficient to chart an infant's progress toward stable suck production [18, 19].

Statistics and Nonlinear Modeling. In order to model and quantify the frequency-modulated (FM) component of the NNS burst, the dependent measure of NNS cycle frequency will be regressed against NNS cycle index for each preterm group using an exponential decay model. NNS burst length will be compared between healthy preterm and RDS preterm infants using a repeated measures analysis of variance (SPSS). Each statistical measure will produce an F-score and is considered significant for P-values less than or equal to 0.05.

3. Results

An example of the basic NNS burst-pause structure sampled from a healthy preterm infant at 34 weeks PMA is shown in Figure 1(a). The initial phase (2-3 cycles) of the burst shows

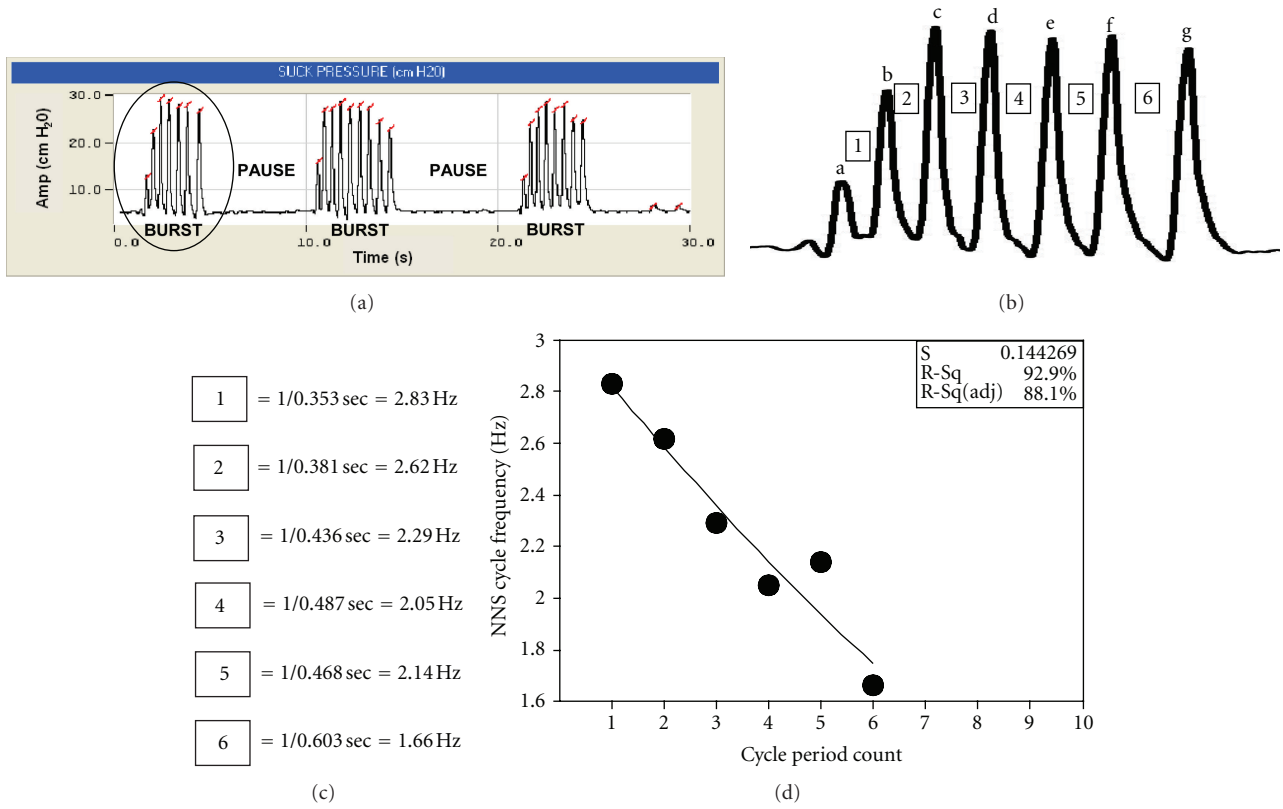

Figure 1: (a) Characteristic burst-pause pattern of NNS. (b) Outset window of the first burst in the 30-second sample. This burst has 7 cycles, identified by letters a–g, and 6 suck cycle periods, identified by numbers 1–6. (c) Individual cycle periods converted to frequency ($1/T$). (d) Plot of cycle period count versus suck cycle frequency (Hz) to demonstrate NNS frequency modulation (FM).

a rapid growth in compression cycle amplitude followed by a slower amplitude decay phase for each of the 3 bursts in this sample. An expanded view of the first NNS burst is shown as an outset pressure waveform in Figure 1(b). In addition to the amplitude modulation (AM), there is evidence of frequency modulation (FM) in Figure 1(c), with the interpeak time intervals translating to a steady decline in cycle frequency from 2.83 Hz (period 1) to 1.66 Hz (period 2). A quadratic regression shows a significant negative relation between NNS cycle frequency and cycle period count with an R^2 of 92.9% for this NNS burst (Figure 1(d)).

The distribution of NNS compression cycle period frequencies is shown for control (Figure 2) and RDS infants (Figure 3). The analyzed data includes a total of 400 NNS bursts (control = 234; RDS = 166), and a boxplot of NNS cycle frequency by period count for each group generally follows a negative exponential decay function. The mean period frequency in each plot is represented by the dotted vertical line. ANOVA revealed a significant difference ($F(1, 398) = 25.63$, $P < 0.0001$) in the mean burst length between the groups with the longest mean burst length (5.67 cycles/burst) belonging to the healthy preterm controls and the shortest mean burst length (3.87 cycles/burst) associated with the RDS infants (Figure 4).

Exponential decay regression analyses for NNS burst cycle frequency as a function of cycle period count revealed

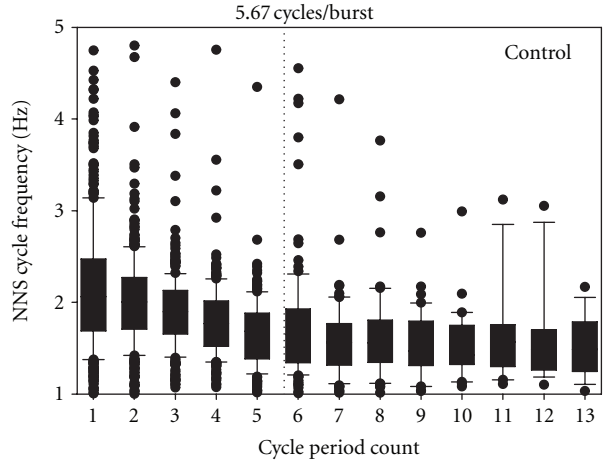

Figure 2: Boxplot of NNS cycle frequency (Hz) as a function of NNS cycle period count for control preterm infants ($N = 17$). Mean NNS burst length is indicated by dotted vertical line.

highly significant negative decay functions for both control and RDS infants. The predicted-Y and 95% confidence intervals (CI) for control and RDS NNS cycle frequency are shown in Figures 5 and 6, respectively. The predicted-Y for healthy control NNS burst cycle frequency was described

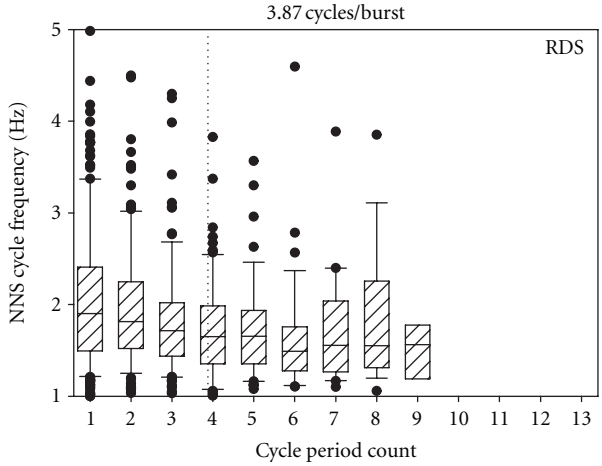

Figure 3: Boxplot of NNS cycle frequency (Hz) as a function of NNS cycle period count for RDS preterm infants ($N = 17$). Mean NNS burst length is indicated by dotted vertical line.

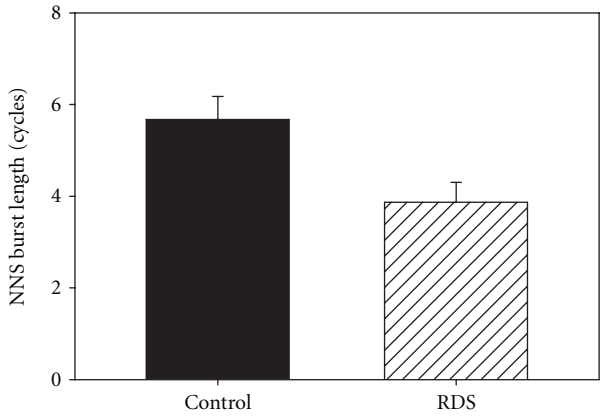

Figure 4: Bargraph of NNS burst length (cycles) for control and RDS preterm infants.

by the equation $Y = 1.3902^{1.9166/x+3.2240}$ ($F = 86.06$, $P < 0.0001$), and the predicted-Y for RDS NNS burst frequency was described by the equation $Y = 1.3082^{3.1253/x+5.4661}$ ($F = 11.80$, $P < 0.0001$), where Y is the NNS cycle frequency and X is the NNS cycle period count. Overall, the variability in NNS cycle frequency is less among control compared to RDS infants as shown by the 95% CIs. As expected, maximum NNS burst length was longer for control (13) versus RDS (9) infants.

The motor gestalt of NNS burst pattern form, quantified as the NNS spatiotemporal index was significantly lower for healthy control (STI = 66.29) versus RDS (STI = 85.44) preterm infants ($F(1, 62) = 24.44$, $P < 0.0001$) (Figure 7). The lower STI observed among control infants reflects a more developed, less variant NNS burst compression pressure pattern compared to their RDS counterparts.

4. Discussion

By 32 weeks PMA, a well-organized NNS burst in a healthy infant consists of approximately 7 cycles at an average

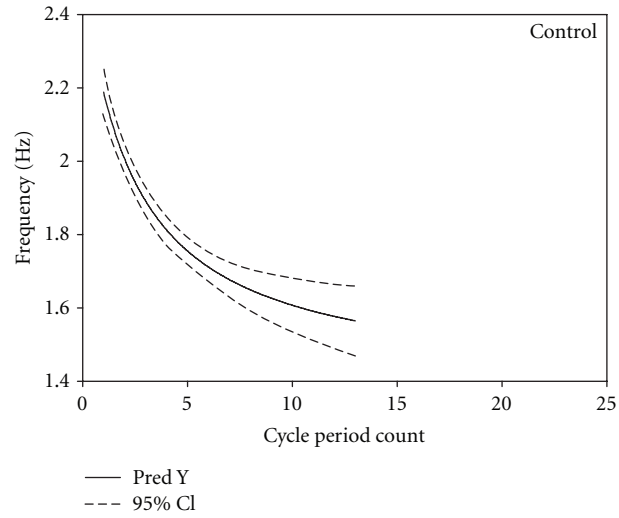

Figure 5: Exponential regression decay function for control NNS burst cycle period frequency by NNS cycle period count. 95% confidence interval given by the dotted line.

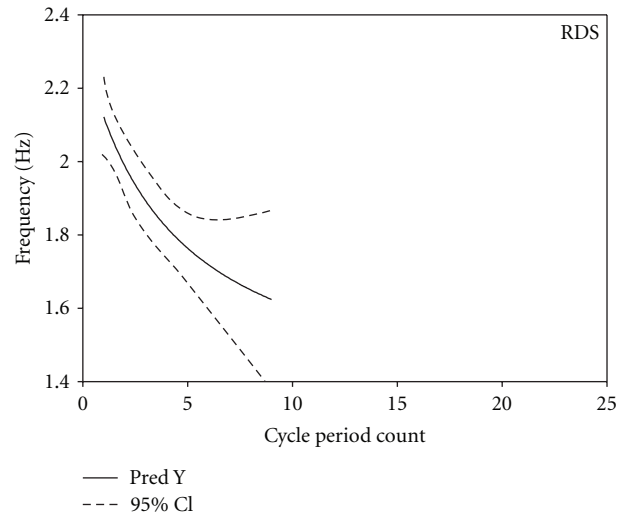

Figure 6: Exponential regression decay function for RDS NNS burst cycle period frequency by NNS cycle period count. 95% confidence interval given by the dotted line.

frequency of 2 Hz, and a mean peak compression pressure of 17 cm H_2O [4, 15, 27, 28]. The present study has detailed a "fine" feature of the NNS burst in healthy preterm infants at 34 weeks PMA, described as an FM or frequency modulated component of NNS burst pattern formation which exhibits a relatively invariant profile with an initial period frequency of 2.2 Hz that decays exponentially to approximately 1.6 Hz by cycle period number 13. This stable pattern of FM modulation exhibited among the 17 healthy preterm controls at 34.11 wks PMA was markedly different for preterm RDS infants who endured more than a month of O_2 supplementation therapy (mean = 34.2 days). For the RDS profile, the FM feature is more variable as evidenced by the spread of the 95% confidence intervals and begins at

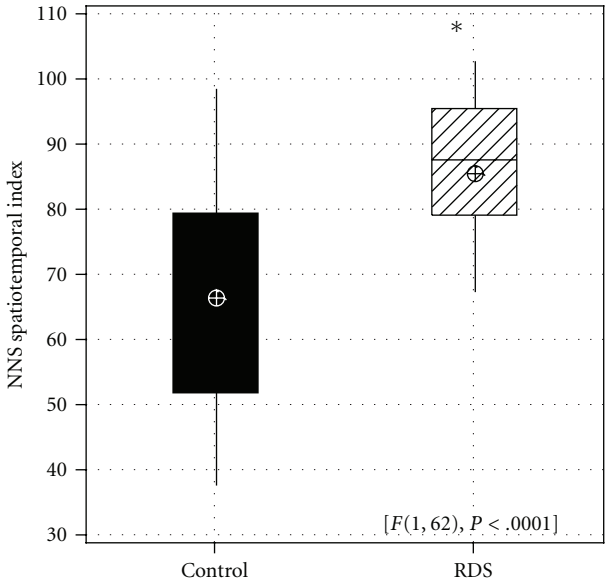

Figure 7: Boxplot of nonnutritive suck spatiotemporal index (NNS STI) for control and RDS preterm infants.

a lower start frequency, with exponential decay over a shorter maximum NNS burst length of 9 cycle periods of 2.2 Hz.

We attribute the reduced NNS burst structure manifest among the sample of 15 RDS infants to an altered and maladaptive experiential set during a critical period of assembly for the central pattern generation and refinement of suck. This includes both sensory deprivation and motor restriction of the orofacial apparatus which contribute to developmental delay in the integrity of the sCPG, ultimately contributing to the delays observed in attainment of independent oral feedings [17]. Development of the central nervous system, including central pattern generators can be modified by environmental factors [27, 28, 34–37]. Lengthy oxygen supplementation procedures in the NICU cost the preterm infant precious sensory and motor experiences during a critical period of brain development when the central patterning of suck and prefeeding skills are being refined. Even the presence of a nasogastric (NG) feeding tube has negative effects on sucking and breathing [38]. Trussing the lower face with poly tubes and tape also restricts the range and type of oral movements and limits cutaneous experiences with the hand and fingers. Interruption of these experiences may impair fragile syntheses of how the brain maps these functions [39, 40]. For some preterm infants, poor suck and oromotor dyscoordination persists well into early childhood and may lead to significant delays in the emergence of other oromotor behaviors, including feeding, babbling, and speech-language production [41, 42]. Failure to establish oral feeding skills in the NICU may result in the infant being sent home on gavage or gastric tube feedings, and hinder the development of oral feeding behaviors. The difficulties associated with establishing oral feed competence along with the additional costs for extended hospitalization underscore the need for precise assessment and therapeutic tools to facilitate the development of normal oral motor skills [11, 12, 43, 44].

Establishing a patterned NNS for the developing infant carries many positive benefits, including growth, maturation, and gastric motility, while decreasing stress [24, 43–49], improving state control before-feeding [45, 47, 50–52] and after-feeding [48], and enhancing oral feeds [17–19, 53]. Use of a pacifier for NNS appears to decrease the frequency of apnea and cyanosis, and improve breastfeeding scores [54]. The NNS accelerates the transition from tube to independent oral feeding and is presumed to enhance the maturation of neural systems responsible for ororhythmic activity [55–57]. The sensory consequences associated with the production of NNS appear to provide beneficial effects on oral feeding performance and the development of specific sucking skills [11, 12]. Accurate assessment of oromotor dyscoordination in the preterm infant extends beyond the immediate issues surrounding the transition to oral feed competency, and may serve as a potent clinical marker for brain development and neurodevelopmental outcomes [20].

Modulation of Biological Rhythms. Frequency modulation of a motor output can result for a number of reasons including metabolic demands, cellular mechanisms, or recruitment of cells. Metabolic demands can force a change in frequency. For example, in heart or respiratory rate, modulation in frequency results when metabolic demands change. An increase in the metabolic demands of the body during exercise results in an increase in the heart and respiratory rates. Related to the modulation of CPGs, Grillner [58] identified the underlying cellular mechanisms involved in these neural circuits to include reciprocal inhibition, mutual excitation, plateau properties, or spike frequency adaptation (Ca^{++}-dependent K^{+} channels). Frequency modulation could be the result of interplay between these cellular mechanisms resulting in a fine-tuned and consistent output so that each burst starts with higher frequency cycles and then decreases as the burst progresses.

Frequency modulation of a motor pattern may also occur as part of a sensory feedback loop providing the sCPG with information about the phase of the motor behavior. For example, in animal studies of gait, the change in frequency was the result of peripheral afferent feedback during both ordinary gait and in tasks that required modulation of the gait [59]. Grillner and Zangger [60] also discovered that a motor pattern could breakdown in the absence of sensory input.

Significance of Frequency Modulation in a Biological System. Physiological systems that typically demonstrate modulation are considered disordered when modulation is diminished or absent [61]. This understanding provides a basis for determining the degree of average modulation in NNS and the extent to which the modulation is influenced by environmental factors, genetic defects, or damage to the central nervous system (CNS). Exploration of knowledge in this area would then provide insight into deficits and potential diagnostic and intervention methods. Animal studies have explored the effects of lesions in different locations of the CNS. For example, motor patterns produced by decerebrate and decorticate cats highlighted the ability to produce

rhythmic behavior in both models, but the inability to modulate in response to the environment or to specific needs of the animal when the lesion eliminated input from the basal ganglia is yet to be highlighted [62]. Understanding more about the components of the sCPG could identify a CNS location that is responsible for the temporal feature of the FM NNS.

5. Conclusion

In summary, healthy preterm infants manifest a significantly longer NNS burst structure when compared to infants with RDS. Second, there is an FM feature of NNS burst formation that is distinctly different for healthy control and RDS infants. Third, healthy preterm infants suck at a higher frequency at the onset of the suck burst when compared with the RDS infants. Finally, for both infant groups, the suck cycle periods increase in duration from burst onset to completion according to an exponential decay function. The ability of practitioners in the NICU to rapidly quantify both the coarse and fine features of NNS is expected to lead to more efficient, physiologically guided interventions to allow the preterm infant to safely advance to independent oral feeding.

Future studies could explore the FM NNS and burst evolution as a result of external stimulation. Such stimulation might include presentation of a pacifier that emulates the NNS in its FM burst-pause characteristics. Such information may be used as a diagnostic tool for identifying aberrant NNS patterns and provide status information on CNS organization in preterm-term populations that have experienced insults such as intraventricular hemorrhage (IVH), mild-moderate white matter injuries (PVL), chronic lung disease, infants of diabetic mothers, infants recovering from cardiac surgery, or genetic anomalies such as Down's syndrome.

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Clinical Study

Intrauterine Growth Restriction and the Fetal Programming of the Hedonic Response to Sweet Taste in Newborn Infants

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Intrauterine growth restriction is associated with increased risk for adult metabolic syndrome and cardiovascular disease, which seems to be related to altered food preferences in these individuals later in life. In this study, we sought to understand whether intrauterine growth leads to fetal programming of the hedonic responses to sweet. Sixteen 1-day-old preterm infants received 24% sucrose solution or water and the taste reactivity was filmed and analyzed. Spearman correlation demonstrated a positive correlation between fetal growth and the hedonic response to the sweet solution in the first 15 seconds after the offer ($r = 0.864$, $P = 0.001$), without correlation when the solution given is water ($r = 0.314$, $P = 0.455$). In fact, the more intense the intrauterine growth restriction, the lower the frequency of the hedonic response observed. IUGR is strongly correlated with the hedonic response to a sweet solution in the first day of life in preterm infants. This is the first evidence in humans to demonstrate that the hedonic response to sweet taste is programmed very early during the fetal life by the degree of intrauterine growth. The altered hedonic response at birth and subsequent differential food preference may contribute to the increased risk of obesity and related disorders in adulthood in intrauterine growth-restricted individuals.

1. Introduction

The fetal origins of adult disease hypothesis states that environmental factors, particularly nutrition, act in early life to program the risks for chronic diseases in adulthood [1]. In particular, intrauterine growth restriction (IUGR) is known to be associated with insulin resistance [2, 3], obesity [4–7], and cardiovascular disease [8, 9] in adult life.

As eating habits can contribute to the development of obesity, diabetes, and cardiovascular disease, one could suggest that persistent nutrient imbalances across the lifespan in people who were born IUGR may explain, at least partially, their increased risk to develop metabolic syndrome later in life. Interestingly, studies from different research groups have shown that IUGR individuals indeed have specific food preferences in adulthood [10–12], naturally choosing to eat more foods rich in carbohydrates and/or fat than non-IUGR individuals. Besides, IUGR girls are more impulsive when facing a sweet reward already at 3 years of age [13]. Therefore, IUGR is associated with changes in feeding behavior and preferences that may promote the metabolic changes previously described in this group.

A possible mechanism by which IUGR could permanently alter an individual's food choices is the programming of the sensitivity to the hedonic signaling (i.e., pleasure) associated with the ingestion of a palatable food. Hedonic sensation is reflected in positive patterns of affective orofacial expressions that are homologous between humans and rodents [14–16]. As explained by Berridge [16], the affective pattern of taste reactivity components reflects palatability or affect more closely than it reflects either ingestion or sensation [17, 18]. It was already shown that prenatal protein

Table 1: Study participants' baseline characteristics according to the solution given.

Sample characteristics	Sucrose ($n = 10$)	Water ($n = 6$)	P
Males (%)	6 (60%)	4 (66.6%)	0.61[§]
Birth weight (g)	1032.50 (810.00; 1320.00)	975.00 (822.50; 1411.25)	0.91[*]
Gestational age (weeks)	28.00 (25.00; 29.0)	27.50 (26.50; 29.00)	0.87[*]
Birth weight ratio	0.99 (0.88; 1.13)	0.92 (0.85; 1.12)	0.75[*]

[*] Mann-Whitney test and [§] Fisher's Exact test. Data are expressed as median (25th percentile; 75th percentile) or n (percentages).

malnutrition changes the response to reward in adult rodents [19], which may suggest that the same phenomena may be happening in humans. This group of evidence prompted us to propose the hypothesis that IUGR leads to fetal programming of the hedonic responses to the sweet taste, and in the current study we aimed at verifying if IUGR would be related to an altered pattern of affective orofacial expressions to the sweet taste very early in life, addressing this question in preterm infants in their first day of life.

2. Subjects and Methods

This study was performed by secondary analyses of nonused data collected for the purpose of a different project. The original protocol was developed with the objective to investigate the efficacy of routine sucrose analgesia for procedural pain in the first week of life in preterm infants, and was described in detail elsewhere [20, 21]. Briefly, a level III university-affiliated NICU in Canada was the site for the study, providing ethics approval by a constituted review board. Infants, who were born between 25 and 31 completed weeks' postconceptional age, were expected to live according to the opinion of the attending neonatologist, were above the fifth percentile weight for gestational age, had intraventricular hemorrhage less than Grade III and no periventricular leukomalacia, were free of major congenital anomalies, and did not require surgery and whose parents consented within 48 hours of birth were included in the study.

Enrolled infants were randomly assigned to the sucrose or water group from a computer-generated schedule. Only the project nurses in each site knew the group assignment; treating clinicians were blind to group assignment. Solutions of 0.1 mL of 24% sucrose or water were drawn up into sterile syringes and placed in the unit medicine refrigerator. Every time the infant was to undergo an invasive (e.g., heel lance, intravenous cannulation, arterial puncture, and injection) or noninvasive but presumably uncomfortable procedure (e.g., endotracheal tube suctioning, tape/lead removal, and gavage insertion for feeding), the solution in the syringe was administered into the infant's mouth 1 minute before the beginning of the procedure.

A small-wide-angle lens camera rested on top of the isolette and was connected to a mat on the floor next to the isolette such that stepping on the mat triggered 5-minute recording. In this way, facial actions could be recorded during painful procedures. While the original study verified the analgesic effect of sucrose in minutes following the procedure, the current study evaluated the hedonic response using the first 15 seconds of facial capture after the sucrose solution was given orally and immediately before the painful procedure. We used filming from the very first time that the newborn received the oral solution, which occurred in the first 24 hours of life for all the subjects in the current study.

The positive hedonic reaction was compiled by adding scores for (a) rhythmic extension of the tongue outwards along the midline, and sometimes upwards, past the outer edge of the lips, often simultaneously accompanied by slight dropping of the jaw; this is followed immediately by retraction of the tongue and closure of the jaw, and the cycle is repeated rhythmically again and again, each cycle lasting 300–1200 ms and (b) lateral nonrhythmic tongue protrusions, which were sweeping extensions of the tongue sideways along the lateral border of the mouth and along the lips on one side of the mouth [15, 22]. A trained observer blinded to the solution given scored the videotapes frame-by-frame (1 frame = 1/10 s) watching the first 15 seconds of shooting after the administration of the oral solution.

The definition of IUGR was based on the birth weight ratio (BWR), which is the ratio between the infant birth weight and the sex-specific mean birth weight for each gestational age for the local population [23, 24]. For this study, BWR was used as a continuous variable reflecting the degree of IUGR for a given infant.

2.1. Statistical Methods. Quantitative variables were described using median (25th percentile; 75th percentile), while categorical data were described using absolute (n) and relative (%) frequencies. To establish potential confounders, children who received sucrose or water were compared on key variables including gender, gestational age, birth weight, and BWR using Mann-Whitney test for quantitative variables and Fisher's Exact test for categorical variables.

Spearman correlation was used to analyze the relation between BWR and the hedonic response according to the solution offered. Statistical significance for all analyses was set at $P < 0.05$.

3. Results

Table 1 depicts the baseline characteristics of subjects receiving the different solutions. There were no statistical differences between children that received water versus sucrose regarding gender, birth weight, gestational age, and birth weight ratio (Table 1).

There was a positive correlation between the BWR used as a continuous variable and the hedonic response to

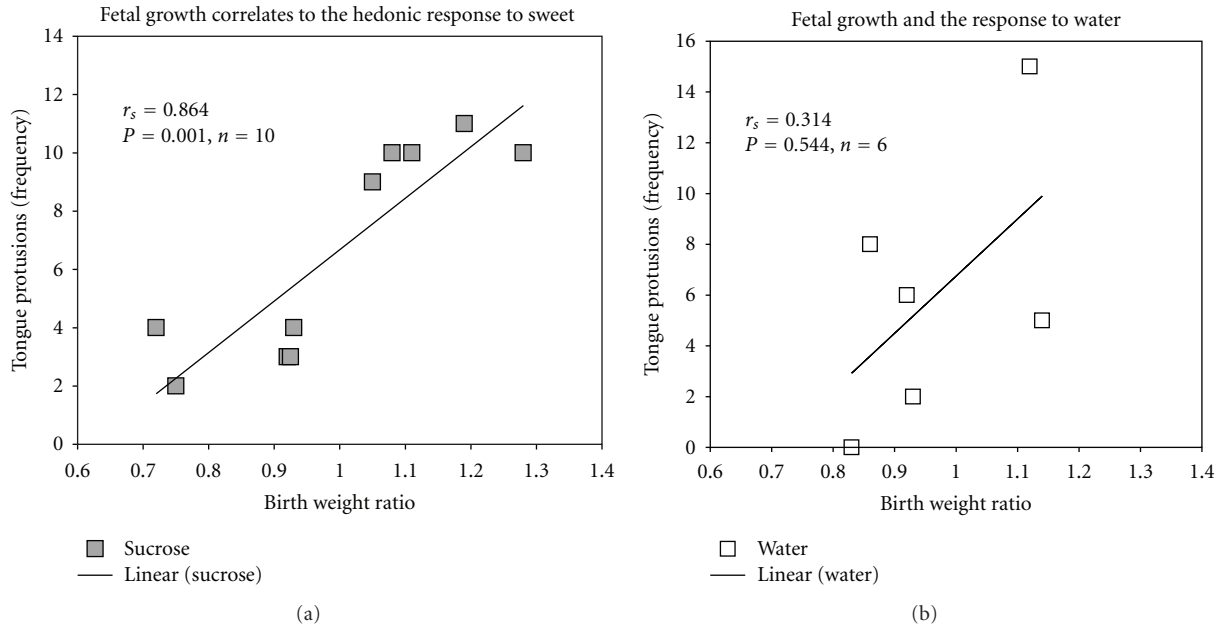

Figure 1: Correlations between birth weight ratio and the frequency of tongue protrusions (hedonic response) to (a) sucrose and (b) water.

the sweet solution in the first 15 seconds after the offer ($r = 0.864$, $P = 0.001$). That is, the greater degree of intrauterine growth restriction, the less the frequency of the tongue protrusions demonstrated after the sucrose offer (Figure 1(a)). There was no correlation between BWR and the hedonic response when the solution given was water ($r = 0.314$, $P = 0.544$) (Figure 1(b)).

4. Discussion

In this study we showed that intrauterine growth correlates with the hedonic response to sweet in preterm infants in their first day of life. In preterm newborns born as early as 27 weeks gestation, the intensity of growth restriction is highly and inversely related to the frequency of positive affective reactions to the sweet taste. One could propose that IUGR leads to a decreased sensitivity to the enjoyment elicited by the sweet taste and would possibly overconsume this and other types of palatable foods when trying to reach a higher degree of pleasure.

Interestingly, the current study agrees with our previous findings [10, 13] as well as with reports from other groups [11, 12] demonstrating that IUGR is indeed associated with an increased consumption of palatable foods at different times during the life-course. At age 24, women born severely growth restricted were shown to prefer to eat more carbohydrates and less protein than women born non-growth restricted, which was accompanied by an increased waist to hip ratio in this group [10]. Besides, people exposed to undernutrition during early fetal stages (the Dutch Famine birth cohort) were more likely to consume a high-fat diet at middle age and had more pronounced hypercholesterolemia, hypertriglyceridemia, and a twofold prevalence of coronary heart disease when compared to nonexposed individuals [11]. In another study involving a different sample of subjects exposed to the Dutch Famine in the first half of gestation, it was shown that they had higher reported absolute intakes of energy, fat, and protein and lower reported absolute intakes of carbohydrate than did the controls at middle age [12].

In addition, we recently showed that among children with normal birth weights, 3-year-old girls show a significantly greater ability to delay responding in a task using sweet as a reward when compared to boys. However, among IUGR girls, this ability to delay responding is lost. Furthermore, this impulsive responding at 36 months in girls predicts both increased consumption of palatable fat at 48 months of age and higher BMIs at 48 months of age [13]. Therefore, it seems that IUGR programs the hedonic response to palatable foods (such as sweet flavor), leading to an altered behavior when facing this type of foods that is seen already in childhood [13] and to an increased consumption in adult life [10–12]. The chronic increased ingestion of these aliments could, in the long term, lead to overweight, atherosclerosis, and cardiovascular disease already described in this group [4–9, 25, 26]. Alternatively, the altered response to the sweet taste in a critical period by itself programs the neural circuits responsible for the establishment of food preferences, leading to altered feeding choices in adult life in IUGR individuals.

Considering that the taste reactivity facial reactions are homologous in humans and animals [15, 16], and therefore share underlying brain circuits, one could argue that IUGR programs the functioning of these circuits during fetal life. Berridge and coworkers have described "hedonic hotspots" in the nucleus accumbens and ventral pallidum that use opioid and endocannabinoid signals to amplify the hedonic response for sweetness [27]. Microinjection of the opioid agonist DAMGO in these areas in rats causes sucrose taste to elicit over twice as many hedonic reactions

as it normally does [27–30]. Similarly, microinjection of anandamide, which likely acts by stimulating the CB1 type of cannabinoid receptor, doubles the number of positive hedonic facial reactions that sucrose taste elicits from rats [29, 31]. Intrauterine growth restriction may decrease the sensitivity of these systems, by diminishing the action of the mu subtype of opioid receptor or the CB1 type of cannabinoid receptor, leading to a decrease in the hedonic response to the sweet taste.

The apparent contradiction of less hedonic response early in life versus increased consumption in adulthood could be explained by a detachment of the normal close association between the hedonic value (or "liking") and the incentive salience (or "wanting"). This could lead to motivated food consumption that is no longer hedonically driven by the activation of mesolimbic dopamine mechanisms of incentive salience, or even opioid circuits outside the hedonic hotspots. The suppression of positive hedonic reward systems or activation of dysphoric stress systems might prompt persistent attempts to use palatable food as a relief [32].

Our study has some limitations. Mainly, the small number of participants did not allow us to employ more adequate statistical adjustments (e.g., considering SES and gender in the analysis) and to classify the newborns into IUGR or non-IUGR categories. In addition, other components of the hedonic reaction could not be scored for the angle of the shooting performed. However, the correlation is very intense for a biological variable, especially considering that the study outline and the selection of participants were not primarily designed to test the current hypothesis.

In conclusion, this is the first evidence of fetal programming of the hedonic response to the sweet taste in humans by IUGR. This takes on added significance considering that these individuals also show a persistent preference for palatable foods later in life [10–12], as well as an increased risk for overweight and related metabolic and cardiovascular consequences [4–9, 25, 26]. Such group of evidence [33] could potentially bring enlightment to future studies aiming primary prevention measures in this population.

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Research Article

Insights into Neonatal Oral Feeding through the Salivary Transcriptome

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Background. The development of safe and effective oral feeding skills in the newborn is complex and may be associated with significant morbidities. Our understanding of neonatal oral feeding maturation at the molecular level is limited, providing an opportunity to utilize emerging molecular techniques to accurately assess neonatal oral feeding skills. *Objective*. To identify key regulatory genes in neonatal saliva involved in successful oral feeding. *Methods*. Previously, our laboratory identified 9,286 genes in saliva that statistically significantly altered their gene expression as premature newborns gained advanced oral feeding skills. In this report, genes previously identified underwent an updated and targeted pathway analysis with Ingenuity Pathway Analysis (IPA) to identify potential candidate genes involved in successful oral feeding. Genes were considered if they were in the five most significantly up- and down-regulated physiological pathways and were associated with the keywords "feeding", "digestion" and "development". *Results*. There were 2,186 genes that met criteria. Pathways associated with feeding behavior, cranial nerve development, and the development of the nervous, skeletal, and muscular systems were highlighted. *Discussion*. These data provide important insights into the biological processes involved in oral feeding in the newborn at a molecular level and identify novel pathways associated with successful oral feeding.

1. Introduction

The vast majority of infants admitted to the neonatal intensive care unit (NICU) must acquire the skills for successful oral feeding prior to discharge. For the preterm infant, this complex task is not without risks. Successful oral feeding involves the maturation and integration of the nervous, sensory, muscular, and digestive systems. Failed oral feeding trials can result in an array of morbidities including choking, aspiration, bradycardia, desaturations, feeding aversions, and both short- and long-term impaired neurological outcomes [1–4]. Further, there is a subset of term infants who have either delayed or unsafe oral feeding skills, resulting in prolonged hospitalization and/or surgical placement of a gastric tube for administration of enteral nutrition. Despite the fact that the vast majority of infants in the NICU are at risk for these morbidities, research on neonatal feeding is relatively scarce compared to other complications of prematurity, such as bronchopulmonary dysplasia [5] and necrotizing enterocolitis [6], that almost exclusively affect a much smaller percentage of infants born at <32 weeks' gestation and/or at extremely low or very low birthweights [7].

Currently, newborns rely on the interpretation of subjective feeding cues by their caregivers to determine when it is safe to orally feed [8, 9]. Our understanding of the complexities of oral feeding maturation in the developing infant at the molecular level is largely unknown. Thus, there is an opportunity to incorporate emerging molecular techniques with conventional clinical approaches to improve our understanding of the complexities of oral feeding, and to develop objective diagnostic assays to accurately assess neonatal oral feeding skills.

Previously, our laboratory described the enormous amount of real-time global developmental information available from premature infants through noninvasive salivary gene expression analyses [10]. Although in the initial study we did not specifically target gene transcripts involved in oral

Table 1: Pertinent clinical information of subjects.

Subject	Gender	Gestational age (weeks)	Birthweight (g)	Medical complications
1	Male	29	1389	Respiratory distress syndrome (RDS), apnea, anemia, and hyperbilirubinemia
2	Female	28 3/7	942	RDS, hyperbilirubinemia, anemia, and apnea
3	Male	28 3/7	1123	RDS, hyperbilirubinemia, apnea, anemia, and retinopathy of prematurity (stage 2, zone 2)
4	Female	32	1683	RDS, hyperbilirubinemia, apnea, and anemia
5	Female	32	1379	RDS, hyperbilirubinemia, apnea, and anemia

feeding, this original discovery-driven research identified key regulatory genes, as well as novel pathways, associated with oral feeding in the preterm infant.

In the two years since this initial work was published, our understanding of the physiological functions of the genes initially identified continues to improve. Newly published reports on gene functions and their association with oral feeding have emerged. This has prompted a targeted reexamination of the data to identify previously unrecognized genes in neonatal saliva that may correlate to oral feeding success. Identifying these genes and their associated biological and physiological pathways may not only lead to objective, noninvasive salivary biomarkers that accurately predict oral feeding readiness, but may also highlight aberrant developmental pathways that correlate with pathological feeding behavior in the newborn.

2. Materials and Methods

This study was approved by the Tufts Medical Center Institutional Review Board. Parental consent was obtained for all enrolled subjects ($n = 5$). Salivary samples were collected and processed as previously described [10]. Gestational age at birth of the subjects ranged from 28 to 32 weeks. There were two females and three males in this data set. Pertinent clinical information, including medical complications, of each subject can be found in Table 1. Salivary samples were collected from each subject from the following time points: (1) no feeds; (2) partial gastric feeds; (3) full gastric feeds; (4) some oral feeds; (5) advanced oral feeds. Salivary RNA was extracted, amplified, and hybridized onto the Affymetrix HG U133 2.0 Plus gene expression arrays.

2.1. Analysis. All arrays ($n = 25$) underwent normalization and bioinformatic analysis as previously described [10]. Genes that were shown to statistically significantly alter their gene expression over time at a false discovery rate P value of <0.05 were identified ($n = 9{,}286$). There were 5,764 up-regulated and 3,522 down-regulated genes that met statistical criteria and altered their expression profile as the subjects matured through the feeding stages. For the purpose of this study, the up- and down-regulated gene lists were uploaded into the newest version of Ingenuity Pathway Analysis (IPA Content version: 11904312, release date 12-15-2011). IPA then performed a functional analysis of the gene lists with a right-tailed Fisher's exact test to calculate a P value determining the probability that each biological function assigned to that data set was due to chance alone. This analysis provided a comprehensive biological assessment of the gene-gene interactions, gene functions and gene regulation in our data sets.

In order to provide a targeted analysis of genes most likely to be related to oral feeding skills, genes that clustered into statistically significantly biological pathways by IPA were only considered if they met the following criteria: (a) they were in the five most statistically significantly up- or down-regulated physiological pathways; (b) they were associated with the key terms "feeding," "digestion," and/or "development" in the IPA analysis. Thus, genes had to statistically significantly alter their gene expression over time, and be identified by IPA as significantly clustering into pathways believed to be associated with oral feeding skills to be considered in this analysis. Genes that met these criteria were further reviewed by the author with the use of IPA, PubMed, and EntrezGene to better understand their functions and possible roles in neonatal oral feeding.

3. Results and Discussion

From 2010 when the data was initially published until present, the functions of 4% of the genes in the original data sets have been modified. As such, those genes are no longer considered valid for previously identified biological functions, canonical pathways, or networks. This recategorization of genes is a direct result of emerging literature and an improved understanding of gene function, gene-gene interaction, and gene regulation. Although it is common for gene expression analyses to change over time, the slight difference in gene analysis from this data set has allowed for the identification of genes not previously known to be associated with neonatal oral feeding. The most significant physiologic pathways identified in this analysis highlight the complexity of oral feeding and involve not only tissue and nervous system development, but key pathways involved in feeding behavior. The five most statistically significantly up-regulated pathways were "behavior" ($10^{-10} < P < 10^{-2}$), "nervous system development" ($10^{-9} < P < 10^{-2}$), "tissue development" ($10^{-7} < P < 10^{-2}$), "embryonic development" ($10^{-7} < P < 10^{-2}$), and "organ development" ($10^{-7} < P < 10^{-2}$). The five most statistically significantly down-regulated pathways were "hematological system development and

Table 2: Genes involved in feeding behavior ($P < 10^{-5}$).

Gene	Gene symbol	Relevant biological function
Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	ACE	This gene encodes an enzyme involved in catalyzing the conversion of angiotensin I into a physiologically active peptide angiotensin II.
Cholecystokinin A receptor	CCKAR	In the central and peripheral nervous system this receptor regulates satiety.
Cannabinoid receptor 1 (brain)	CNR1	Animal experiments utilizing receptor antagonists resulted in suppressed food and water intake with concurrent decreased body weight.
Corticotropin releasing-hormone	CRH	Corticotropin-releasing hormone is secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress.
Corticotropin releasing-hormone receptor 1	CRHR1	The encoded protein is essential for the activation of signal transduction pathways that regulate diverse physiological processes including obesity.
Diencephalon/mesencephalon homeobox 1	DMBX1	This gene is known to be involved in adult feeding behavior and may play a role in brain and sensory organ development.
Free fatty acid receptor 1	FFAR1	The encoded protein is a receptor for medium and long chain free fatty acids and may be involved in the metabolic regulation of insulin secretion.
Glutamate decarboxylase 2 (pancreatic islets and brain, 65 kDa)	GAD2	This gene has been shown to be a candidate gene for obesity in humans.
Galanin-like peptide	GALP	This gene is involved in biological processes including hypothalamic regulation of metabolism.
Galanin receptor 3	GALR3	The neuropeptide galanin modulates a variety of physiologic processes including feeding behavior.
Glucagon	GCG	Glucagon is a pancreatic hormone that counteracts the glucose-lowering action of insulin by stimulating glycogenolysis and gluconeogenesis.
Growth hormone secretagogue receptor	GHSR	The encoded protein may play a role in energy homeostasis and regulation of body weight.
Glucagon-like peptide 1 receptor	GLP1R	This gene is involved in energy reserve metabolic processes and feeding behavior.
Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	GRIN2B	NMDA receptor channel is involved in the activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning.
Hypocretin (orexin) receptor 2	HCRTR2	The protein encoded by this gene is a G protein coupled receptor involved in the regulation of feeding behavior.
Histamine receptor H3	HRH3	This gene encodes an integral membrane protein and can regulate neurotransmitter release.
5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled	HTR1A	Gene has been shown to be involved in control of food intake in obese rats.
5-hydroxytryptamine (serotonin) receptor 2C, G protein-coupled	HTR2C	This gene is involved in feeding behavior.
Interleukin 1 receptor antagonist	IL1RN	The protein encoded by this gene is a member of the interleukin 1 cytokine family.
Janus kinase 1	JAK1	Knockout mice of this gene exhibit decreased nursing behavior.
Junctophilin 1	JPH1	This gene is involved in muscle organ development.
Lactalbumin, alpha	LALBA	This gene encodes alpha-lactalbumin, a principal protein of milk.
Leptin receptor	LEPR	This protein is a receptor for leptin and is involved in the regulation of fat metabolism.
Melanin-concentrating hormone receptor 1	MCHR1	The gene is involved in the neuronal regulation of food consumption.
NK2 homeobox 1	NKX2-1	This gene is involved in brain development and feeding behavior.
Neuropeptide Y receptor Y1	NPY1R	Neuropeptide Y exhibits a diverse range of important physiologic activities including regulation of food consumption.
Neuropeptide Y receptor Y2	NPY2R	This gene is involved in regulating feeding behavior.
Neurotrophic tyrosine kinase, receptor, type 2	NTRK2	This gene is involved in feeding behavior. Mutations in this gene have been associated with obesity.
Opioid receptor, kappa 1	OPRK1	This gene is involved in regulating behavior.
Peroxisomal biogenesis factor 13	PEX13	This gene is involved in suckling behavior.
POU class 4 homeobox 1	POU4F1	This gene is highly expressed in the developing sensory nervous system.

Table 2: Continued.

Gene	Gene symbol	Relevant biological function
Prolactin releasing hormone	PRLH	This gene is involved in feeding behavior and regulates multicellular organism growth.
Prostaglandin E receptor 3 (subtype EP3)	PTGER3	This receptor may have many biological functions, which involve digestion and the nervous system.
PTK2 protein tyrosine kinase 2	PTK2	This gene plays a role in glucose response, fat-cell differentiation, and the growth hormone receptor signaling pathway.
Peptide YY	PYY	This gene is involved in digestion and feeding behavior.
Solute carrier family 18 (vesicular monoamine), member 2	SLC18A2	This gene is involved in glucose homeostasis and response to starvation.
Solute carrier family 27 (fatty acid transporter), member 5	SLC27A5	This gene is involved in digestion.
Tachykinin receptor 1	TACR1	This gene is involved in eating behavior.
Tyrosine hydroxylase	TH	This gene plays a role in eating behavior.
Thyrotropin-releasing hormone	TRH	This gene plays a role in eating behavior.
Transient receptor potential cation channel, subfamily M, member 5	TRPM5	This gene plays an important role in taste transduction.

function" ($10^{-10} < P < 10^{-3}$), "hematopoiesis" ($10^{-10} < P < 10^{-3}$), "lymphoid tissue structure and development" ($10^{-9} < P < 10^{-5}$), "organismal survival" ($10^{-9} < P < 10^{-4}$), and "cell-mediated immune response" ($10^{-8} < P < 10^{-5}$). Each pathway is inclusive of related subcategories. For example, within nervous system development, the subcategories "development of cranial nerve" ($P < 0.001$) and "development of olfactory receptors" ($P < 0.01$) were found. There were 1,807 up-regulated genes that met the search criteria; 379 down-regulated genes were also considered.

One of the most novel aspects of both the current and previous analysis is the prominent role of "behavior" in neonatal oral feeding. Of all the statistically significantly upregulated pathways identified, genes associated with "behavior" were the most significant. Within this pathway, a subcategory entitled "feeding" was highlighted ($P < 10^{-5}$). Genes within this pathway were associated with "hyperphagia," "satiety," "obesity," and "weight gain" (Table 2). This novel pathway suggests that oral feeding in the newborn is neither merely reflexive nor solely dependent upon oral musculature and nervous system development. Rather, newborns rely, in part, on complex neurological signaling related to hunger, satiety, and energy expenditure for successful oral feeding. This makes biological sense. During the first year of life, a healthy term newborn will gain 200% or more of its birth weight. A preterm infant may gain upwards of 300% of his or her birth weight. The enormous amount of caloric intake required for such exponential growth is unique to the newborn period of the human lifespan. Thus, it is not surprising that the most statistically significant pathway in our analysis is related to biological mechanisms driving feeding behavior.

In the five most significantly up-regulated physiological pathways, new gene functions have also emerged. For example, in the prior study, the development of only the trigeminal nerve (Cranial Nerve [CN] V) was highlighted. At the time, we speculated that the development of this nerve, which provides motor innervation to the muscles of mastication, was essential for proper coordination of the suck- and swallow-reflex. In this updated analysis, we not only see upregulation of genes involved in the development of the trigeminal nerve, but also have identified genes involved in the developing facial (CN VII) and glossopharyngeal (CN IX) nerves (Table 3). Each of these nerves is known to be essential for safe coordination of swallowing with respiration, with CN VII innervating the sensory component of the facial mask, and CN IX providing sensory taste fibers to the posterior tongue. In addition, the subcategory "development of cranial nerve" was new to this analysis. Here, genes associated with the oculomotor (CN III) and vestibulocochlear (CN VIII) nerves were identified. Sensory development was also prominent in this targeted reanalysis of the data. There is an upregulation of genes involved in the developing eye ($P < 0.01$) and ear ($P < 0.02$), as well as the olfactory system ($P < 0.01$). Genes involved in olfactory receptor development were significantly upregulated as infants matured and learned to orally feed. This, too, is of great interest as emerging literature supports the role of the infant's olfactory system in the establishment of successful breastfeeding [11].

Genes involved in the developing nervous, skeletal and muscle systems were also highlighted in this analysis. There was a wide range of nervous system functions including the development of the brain, spine, central nervous system, neurons, neurites, and ganglions. Development of striated muscle ($P = 0.009$) was significantly upregulated over time, as was developing bone ($P = 0.006$) and cartilage ($P = 0.005$). One of the limitations of this study is that neonatal subjects were followed over several weeks while in the NICU. During that time, there were global developmental processes occurring as the infants matured. However, by limiting salivary collection to five predefined feeding stages, there

Table 3: Cranial nerve development and morphogenesis ($10^{-3} < P < 10^{-2}$).

Gene	Symbol	Relative biological functions
Glossopharyngeal morphogenesis and development ($10^{-3} < P < 10^{-2}$)		
Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	SEMA3D	Nervous system development
Plexin A4	PLXNA4	Cranial nerve morphogenesis; facial nerve morphogenesis (CN VII); glossopharyngeal nerve (CN IX) morphogenesis
Homeobox D3	HOXD3	Glossopharyngeal nerve (CN IX) morphogenesis
Homeobox B3	HOXB3	Glossopharyngeal nerve (CN IX) morphogenesis
Survival and development of trigeminal nerve and ganglion ($10^{-3} < P < 10^{-2}$)		
B-cell CLL/lymphoma 2	BCL2	Neurodegeneration
Glial cell derived neurotrophic factor	GDNF	Axon guidance; neuron differentiation; neuron projection development
Neurturin	NRTN	Neuron projection development; axon guidance
POU class 4 homeobox 1	POU4F1	Neuron differentiation
POU class 4 homeobox 2	POU4F2	Trigeminal nerve development (CN V); suckling behavior
Solute carrier family 6 (neurotransmitter transporter, creatine), member 8	SLC6A8	Neurotransmitter transport
Noggin	NOG	Axon guidance; face morphogenesis; regulation of neuron differentiation
Cranial nerve development and morphogenesis ($P < 10^{-3}$)		
GLI family zinc finger 3	GLI3	Optic nerve morphogenesis (CN II); palate development; tongue development
Cholinergic receptor, nicotinic, beta 2 (neuronal)	CHRNB2	Conditioned taste aversion; optic nerve morphogenesis; vestibulocochlear nerve development (CN VIII)
Chromodomain helicase DNA binding protein 7	CHD7	Face development; nose development; palate development; sensory perception of sound; development in camera-type eye
Hairy and enhancer of split 1, (Drosophila)	HES1	Oculomotor nerve development (CN III); pharyngeal system development; auditory receptor cell differentiation and determination; cochlea development
Neuropilin 2	NRP2	Semaphorin-plexin signaling pathway
Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D	SEMA3D	Nervous system development
Homeobox B3	HOXB3	Glossopharyngeal nerve (CN IX) morphogenesis
Homeobox D3	HOXD3	Glossopharyngeal nerve (CN IX) morphogenesis
Plexin A4	PLXNA4	Cranial nerve morphogenesis; facial nerve morphogenesis (CN VII); glossopharyngeal nerve morphogenesis (CN IX)
Thyroid hormone receptor, beta	THRB	Sensorineural hearing loss
v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	ERBB4	Central nervous system morphogenesis and olfactory bulb interneuron differentiation
Homeobox D3	HOXD3	Glossopharyngeal nerve morphogenesis (CN IX)
POU class 4 homeobox 1	POU4F1	Neuron differentiation
Myosin VA (heavy chain 12, myoxin)	MYO5A	Visual perception
Sal-like 1 (Drosophila)	SALLI	Olfactory bulb development and interneuron differentiation; outer ear morphogenesis

was an opportunity to capture, in real-time, gene expression changes directly related to the attainment of safe oral feeding skills. These data support the complexity of oral feeding and further demonstrate how multiple processes involved in oral feeding may be monitored simultaneously in the newborn at the molecular level.

Of note, statistically significant down-regulated genes were related only to the developing hematological and lymphoid system. There was no obvious link between lymphoid and/or hematological development and oral feeding success that appeared when reviewing the down-regulated pathways and their associated subcategories. While it is possible that the development of the gastrointestinal immune system plays an important role in readiness to feed, our current understanding of these genes prohibits further speculation on their role, if any, in neonatal oral feeding skills.

This research demonstrates that with the advent of recent technical advances and high-throughput screening methods,

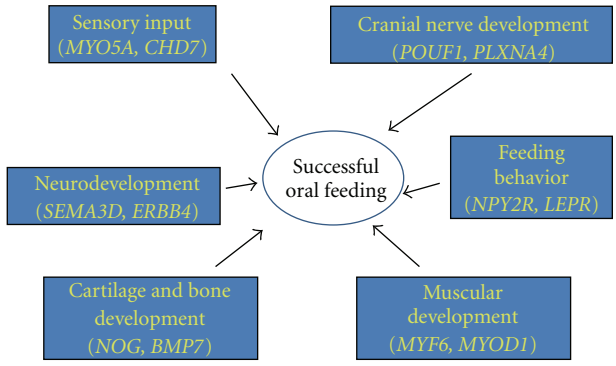

Figure 1: Through salivary gene expression analyses, genes involved in multiple developmental systems that are required for successful neonatal oral feeding can be monitored noninvasively and simultaneously. Combining gene targets, such as those identified, may ultimately lead to a noninvasive, objective, and accurate salivary diagnostic platform to determine readiness to orally feed in the newborn.

we are able to monitor, in real-time, normal and aberrant developmental processes occurring in the newborn from mere drops of saliva. Utilizing this information for the development of noninvasive salivary diagnostic panels is a novel and exciting aspect of translational medicine. Indeed, salivary diagnostic platforms are currently in development for adult patients with oral cancer, breast cancer, Sjögren's disease, pancreatic cancer, melanoma, nonsmall cell lung cancer, acute myocardial infarction, diabetes, and ovarian cancer [12–17]. These platforms are designed to be utilized at the bedside in order to give an accurate diagnosis within minutes with the use of point-of-care technology currently in development [18]. While these platforms are clinically important, none targets a neonatal or even a pediatric patient population. Yet neonates, with their limited blood volumes and clinical fragility, are the ideal patient population on which to apply this technology.

Compared to our prior analysis which examined global developmental processes in the newborn, the present study focused solely on genes believed to be involved in the attainment of successful oral feeding skills. This targeted analysis of the data is laying the foundation for the development of a neonatal oral readiness to feed salivary diagnostic platform. For example, our laboratory recently determined that salivary detection of one of the genes identified in this data set, neuropeptide Y2 receptor (*NPY2R*), a known hypothalamic regulator of feeding behavior, has a 95% positive predictive value for immature oral feeding skills [19]. A limitation to this biomarker is that if undetected in saliva, it is only 27% accurate in determining a safe and effective oral feeding pattern. However, as this work moves forward, other genes identified in this targeted analysis could be incorporated into a comprehensive platform, not only for the development of an accurate and objective diagnostic assay, but also to improve our understanding of aberrant feeding patterns in the newborn (Figure 1).

4. Conclusion

Neonatal salivary transcriptomic analysis provides noninvasive and objective information about the learning process of oral feeding in the newborn at the molecular level. These data further confirm the complexities of oral feeding and suggest that the development of feeding behavior is a novel and essential biological component to successful oral feeding. This research lays the foundation for the development of an objective, noninvasive assay for the determination of readiness to feed in the neonatal population in order to reduce morbidities and improve care and outcomes.

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Research Article

Oxygen Saturation and Suck-Swallow-Breathe Coordination of Term Infants during Breastfeeding and Feeding from a Teat Releasing Milk Only with Vacuum

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Background. Vacuum is an important factor in milk removal from the breast, yet compression is the predominant component of milk removal from bottle teats. Since bottle-feeding infants have lower oxygen saturation, vacuum levels, and different suck-swallow-breathe (SSwB) coordination to breastfeeding infants, we hypothesised that when infants fed from a teat that required a vacuum threshold of −29 mmHg for milk removal, that oxygen saturation, heart rate, and suck-swallow-breathe (SSwB) patterns would be similar to those of breastfeeding. *Study Design*. Infants ($n = 16$) were monitored during one breastfeed and one feed from the experimental teat. Simultaneous recordings were made of oxygen saturation, heart rate, vacuum, tongue movement, respiration, and swallowing. *Results*. There were no differences in oxygen saturation and heart rate between the breast and the teat. Infants displayed fewer sucks and breaths per swallow during nutritive sucking (NS) compared to non-nutritive sucking (NNS). The number of sucks per breath was similar for NS and NNS although respiratory rates were slower during NS. These patterns did not differ between the breast and the teat. *Conclusion*. These results suggest that vacuum may be conducive to safe and coordinated milk removal by the infant during both breast and bottle-feeding.

1. Introduction

Infant's coordination of the suck-swallow-breathe (SSwB) reflex is integral to safe, efficient, and effective breastfeeding. Healthy term breastfeeding infants are able to simultaneously suck and breathe and to suck and swallow but must briefly stop breathing to swallow, all while maintaining high blood oxygenation [1]. In contrast, during bottle-feeding infants often exhibit lower oxygen saturation than breastfeeding, periods of desaturation, and alternating periods of sucking and breathing [2–5]. It is often assumed that SSwB coordination during breastfeeding is similar to that of bottle-feeding despite some teats having large venting holes, rapid milk flow and high compressibility, whereas on the breast there is variable milk flow and limited compressibility [2, 3, 5–7]. These differences suggest bottle teat design may influence the mechanism by which an infant removes milk [2–4].

The level of intraoral vacuum applied by the infan is important for removal of milk from the breast [8]. Geddes et al. [9] showed that when the infant's tongue was in apposition with the palate, infants held a vacuum on average at −64 mmHg (baseline vacuum), and when the tongue lowered, vacuum increased in strength on average to −145 mmHg (peak vacuum) and milk flowed into the intra-oral cavity. This demonstrated that milk was removed using vacuum rather than compression of the nipple. In contrast, some bottle-feeding studies have demonstrated that infants do not require vacuum to obtain milk [10], which supports the theory that compression of the nipple and/or positive pressure is instrumental in milk removal [11, 12]. During bottle-feeding, infants have shown longer suck bursts [13], disorganised swallowing patterns, and lower oxygen saturation compared to breastfeeding [5]. Thus, in

spite of the importance of vacuum in milk removal, how vacuum influences oxygen saturation, heart rate, and SSwB patterns during breastfeeding and bottle-feeding is not well understood.

We hypothesized that when using only vacuum to remove milk from a teat, infants would show safe and well-coordinated patterns similar to breastfeeding. Therefore, an experimental teat was designed to release milk only when the infant applied a vacuum and used a similar tongue movement to breastfeeding. Geddes et al. [14] have confirmed that breastfeeding infants were able to successfully remove milk from the experimental teat using only vacuum (as opposed to compression). To verify that vacuum enabled the infant to control milk removal in a safe and coordinated manner, we measured oxygen saturation, heart rate, and SSwB patterns simultaneously on the breast and experimental teat.

2. Materials and Methods

2.1. Participants. Mother-infant dyads were recruited through the Child and Adolescent Community Health Service (Oceanic Area Health Service), Perth, WA and via email notification at The University of Western Australia. Infants were healthy, full term, and without feeding difficulties, oral abnormalities (such as ankyloglossia or cleft-lip/palate), or illness. Mothers were breastfeeding and occasionally feeding their infant expressed breast milk via a bottle. Mothers supplied written informed consent to participate in the study, and ethics approval was obtained from the Human Research Ethics Committee at The University of Western Australia.

2.2. Protocol. Participants completed two visits to the research laboratory at the Breastfeeding Centre of Western Australia, King Edward Memorial Hospital, Perth. Each infant was monitored for a breastfeed during one visit and a feed using the experimental teat during the other visit. Simultaneous recordings were made of intraoral vacuum, tongue movement, respiration, oxygen saturation, and heart rate for the entire feed using a customised computerized data collection system (LactaSearch, Medela AG, Baar, Switzerland).

2.3. Feeding Assessments

2.3.1. Suck-Swallow-Breathe, Oxygen Saturation, and Heart Rate Monitoring. Submental imaging of the infant's intra-oral cavity was used to determine both tongue action and milk flow during all monitored feeds using a Toshiba SSA-770A/80, Aplio 80 (Tokyo, Japan) ultrasound machine with an endocavity convex transducer (PVT-661VT) and Parker Ultrasonic Gel (Fairfield, NJ, USA). This method enables both a clear view of the nipple, tongue, hard palate, soft palate, and milk flow into the intra-oral cavity [9, 14, 15]. Intra-oral vacuum was measured using a small silicone tube (Supplemental Nursing System, Medela AG, Baar, Switzerland) filled with sterile water [9, 14, 16]. One end was placed alongside either the mother's nipple or the teat and extended 1-2 mm beyond the tip, and the other end was attached via a silicone tube (650 mm $\times$ 4 mm) and a three-way tap to a pressure transducer (SP854, Memscap, Bernin, France) with disposable clip-on dome (MLA844, AD Instruments, Castle Hill, Australia) [9, 14, 16].

Patterns of respiration and swallowing were measured using respiratory inductive plethysmography (RIP) (Respitrace QDC, SensorMedics, Yorba Linda, CA, USA) from two bands, one placed around the thorax at the level of the nipples and a second around the abdomen at the level of the umbilicus. The output displayed the thoracic trace, the abdominal trace, and the sum of thoracic and abdominal effort. Bands were secured using micropore tape and were connected to the Respitrace. RIP has been validated against ultrasound as a highly reliable method for identifying swallows during breastfeeding [17] and has been used successfully to compare respiratory changes for breast and bottle-feeding in term infants [3, 5]. With RIP and other methods used to detect swallowing, degradation of the signal can occur due to excessive movement of the infant. Alternative methods used for swallowing detection during feeding are invasive and therefore risk interfering with breastfeeding. Thermistors detect changes in nasal temperature, however poor positioning of the sensor, differences in sensors and ambient air temperature often degrade the resulting signals. Pharyngeal pressure monitoring via an intranasal catheter is both invasive and may interfere with respiration during breastfeeding [17, 18]. Taking into account the limitations of RIP, any unsettled feeding/infant movement that altered the signal was noted during recording. Oxygen saturation and heart rate were recorded using pulse oximetry (Radical/MasimoSET V4.1) with a paediatric sensor (LNOP YI Multisite) taped to the distal end of the infant's foot. Outputs from the ultrasound machine, pressure transducer, RIP, and pulse oximeter were synchronised by the Lactasearch and recorded using the software package DIAdem (version 11.1, National Instruments, TX, USA) with a custom designed program for offline data analysis. Milk intake was measured by test weighing infants on an electronic scale (BabyWeigh Scale, Medela AG, Baar, Switzerland; resolution 2 g, accuracy $\pm$0.034%) before and after each feed.

2.3.2. Experimental Teat . The experimental teat was comprised of 3 parts: the hollow silicone top, the base, and a middle control component that regulated milk flow, depending on the level of vacuum applied by the infant and the size of the flow hole. At a threshold vacuum of -29 mmHg, the circular membrane deformed to allow milk flow through a channel at the side of the membrane. The infant was unable to remove milk using only compression of the teat by the jaw/tongue. The base included a duck-bill valve that vented the bottle (Figure 1).

2.4. Data Analysis

2.4.1. Suck, Swallow, and Breathe Variables. The customised script for DIAdem software (National Instruments) was used to extract intra-oral vacuum levels, respiration, swallow, oxygen saturation and heart rate measurements. Each feed

Figure 1: The experimental teat was comprised of 3 parts: (1) silicone teat, (2) middle teat base with raised support areas for the teat and a milk flow component, and (3) base contained a duck bill valve to vent the bottle.

was divided into suck bursts and pauses. Suck bursts were identified as the tongue moving on ultrasound and an active vacuum curve present and pauses as the tongue resting on ultrasound and a stable vacuum trace. Sucks were classified as nutritive sucking (NS) if milk flow was observed in the intra-oral cavity on ultrasound where the milk bolus appeared as a hypoechoic (black) area filled with echogenic white flecks (milk fat globules), nutritive pausing (NP) if the pause occurred directly after NS, non-nutritive sucking (NNS) if no milk flow was observed in the intra-oral cavity on ultrasound (Figures 2 and 3), and non-nutritive pausing (NNP) for subsequent pauses. Ultrasound has been used previously to identify milk flow (milk fat globules) during a suck cycle and suck burst [9, 14, 15]. A breath was defined by visualisation of both an inspiration detected as an increase in voltage, and expiration as a decrease in voltage. A swallow was identified as a stable signal on the trace [17].

For each NS and NNS burst across the entire feed on both the breast and teat, peak vacuum (mean minimum pressure, mmHg), baseline vacuum (mean maximum pressure; mmHg), mean vacuum (mmHg), suck rate (sucks/min), respiratory rate (breaths/min) and suck burst duration were calculated. For each pause across the entire feed, pause type (NP/NNP), mean vacuum, respiratory rate, and pause duration were calculated. Suck bursts and pauses were sequentially numbered to allow analysis of patterns across the feed. Nutritive transfer rates were calculated as the total milk transferred divided by the total duration of NS. Mean, minimum, and maximum oxygen saturation and heart rate were calculated for the entire feed.

For the feeds on the breast/teat, the first three well-visualised NS and NNS bursts were selected and the number of sucks (S), swallows (Sw), and breaths (B) were counted. From this, SSwB ratios were determined by calculating the ratios of S : Sw and B : Sw relative to 1 swallow, and S : B relative to 1 breath.

2.5. Statistical Analysis. Data analysis was performed using R 2.9.0 (The R Core Team) [19]. Packages nlme, [20], lattice [21], and multcomp [22] were used for linear mixed models, graphical exploration, and general linear hypothesis tests, respectively. Differences were considered significant when $P < 0.05$. Summary data is presented as mean ± SD or median (interquartile range).

Feeding characteristics, oxygen saturation, and heart rate for the two feeds were compared using paired Student's t-tests following testing for normality using the Shapiro test or the Wilcoxon rank sum test otherwise. All other variables; the number of sucks, swallows, and breaths; SSwB ratios; sucking rates, respiratory rates; burst duration and burst vacuum levels, were compared using linear mixed models to account for the repeated measures in each feed. Models included individual intercepts as the random effect. Models were selected using forward stepwise regression using a $P < 0.05$ threshold. All nonsignificant predictors and interaction were omitted from the final models unless they were included in a higher level interaction. For all models, an interaction term for feed (breast/teat) type and burst type (NS/NNS/NP/NNP) or (NS/NNS) for sucking variables was included. To determine patterns across the feed for the variables suck rate; respiratory rate; mean/peak/baseline vacuum; burst order was considered as an additional interaction term. Relationships between mean, peak, and baseline vacuum with suck rate and respiratory rates were also tested by adding suck rate and respiratory rate as an additional interaction term. To determine the differences in vacuum level and burst duration between the feed and burst types, Tukey's multiple comparisons of means were made separately for each combination of vacuum level and burst duration for feed type (breast/teat) and burst (NS/NNS/NP/NNP) type.

3. Results

3.1. Feed Characteristics. Eighteen mother/infant dyads were recruited. Two of the eighteen infants refused the teat, one of whom had a breastfeed recorded. Therefore, 16 infants with complete breastfeed and teat data were included in the analysis. At the first study session eleven infants were breastfed and six infants were fed using the teat. Infants were (mean ± sd) 49.4 ± 19.9 days old at the breastfeed and 56 ± 18.3 days old when they fed from the teat. Of the teat feeds, 14 were given by the mother, and the remainder were given by either a family member or a researcher. Milk intake during the monitored feed was significantly higher from the breast ($P = 0.013$); however, nutritive transfer rate ($P = 0.59$), feed duration ($P = 0.25$), and the duration of NS ($P = 0.93$) were not different between the breast and the teat (Table 1).

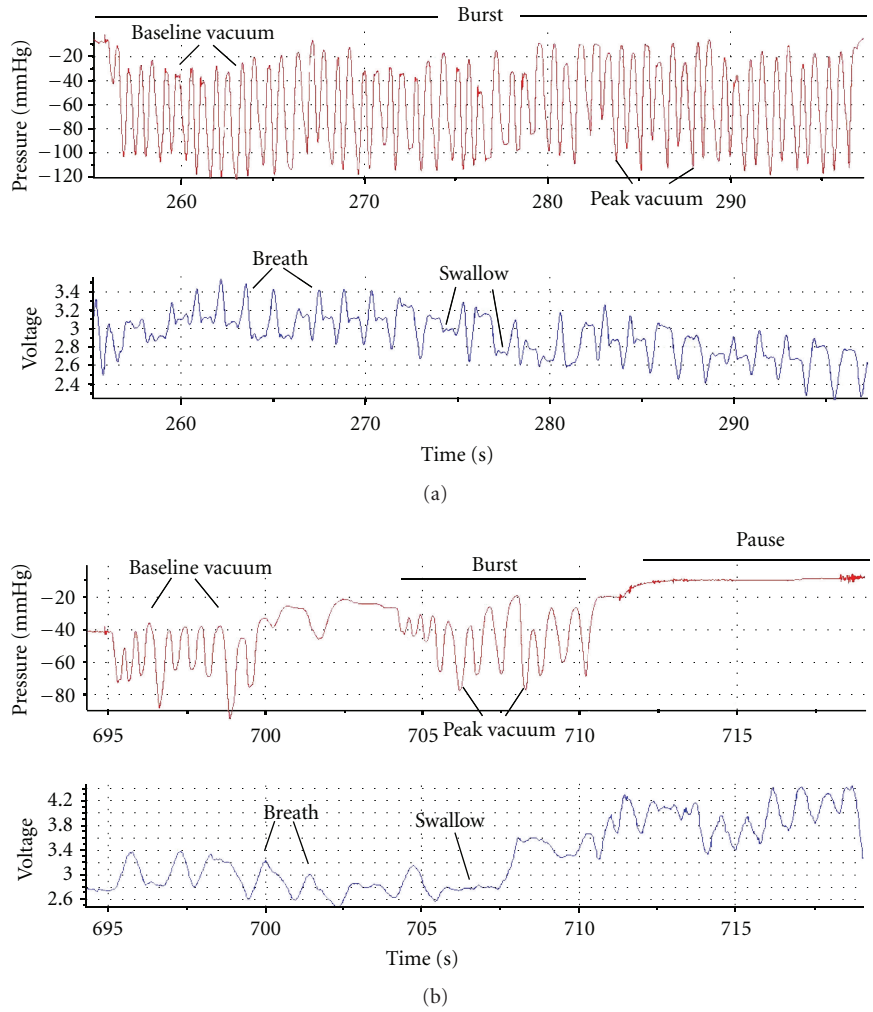

Figure 2: Trace of a suck-swallow-breathe burst of an infant during (a) nutritive sucking (NS) and (b) non-nutritive sucking (NNS) during the monitored breastfeed. During the NS burst, there are more sucks, swallows, and breaths, and the burst is longer than NNS.

Table 1: Feeding characteristics for the monitored breastfeed and the feed from the experimental teat.

Feeding characteristics	Breast	Teat	*P* value
Milk intake (g)	93 ± 36	62 ± 30	0.013
Feed duration (s)	626 ± 173	738 ± 336	0.25
NS duration (s)	263 (212, 373)	264 (128, 318)	0.93
Nutritive transfer rate (g/minute)	23.6 ± 14.8	20.3 ± 13.5	0.59

Results are mean ± SD or median (interquartile range).
NS: nutritive sucking.

Table 2: Oxygen saturation and heart rate for the monitored breastfeed and the feed from the experimental teat.

		Breast	Teat	*P* value
Oxygen saturation (%)	Mean	98.6 ± 1.1	98.6 ± 1.2	0.13
	Minimum	92.9 ± 4.9	94.9 ± 6.5	0.81
	Maximum	99.9 ± 0.3	99.9 ± 0.3	0.33
Heart rate (bpm)	Mean	160.7 ± 10.7	162.5 ± 12.3	0.56
	Minimum	139.0 ± 9.0	142.8 ± 13.1	0.41
	Maximum	178.2 ± 13.0	182.0 ± 18.2	0.43

Results are mean ± SD.

3.2. Oxygen Saturation and Heart Rate. No difference was seen in mean ($P = 0.13$), minimum ($P = 0.81$), and maximum ($P = 0.33$) oxygen saturation or mean ($P = 0.56$), minimum ($P = 0.41$), and maximum ($P = 0.43$) heart rate between feeds from the breast and the teat (Table 2).

3.3. Number of Sucks, Swallows, and Breaths. During NS the number of breaths ($P = 0.03$) and swallows ($P < 0.001$) per burst was higher than the number during NNS. These patterns were similar between the breast and teat (breaths $P = 0.7$, swallows $P = 0.38$). The total number of sucks

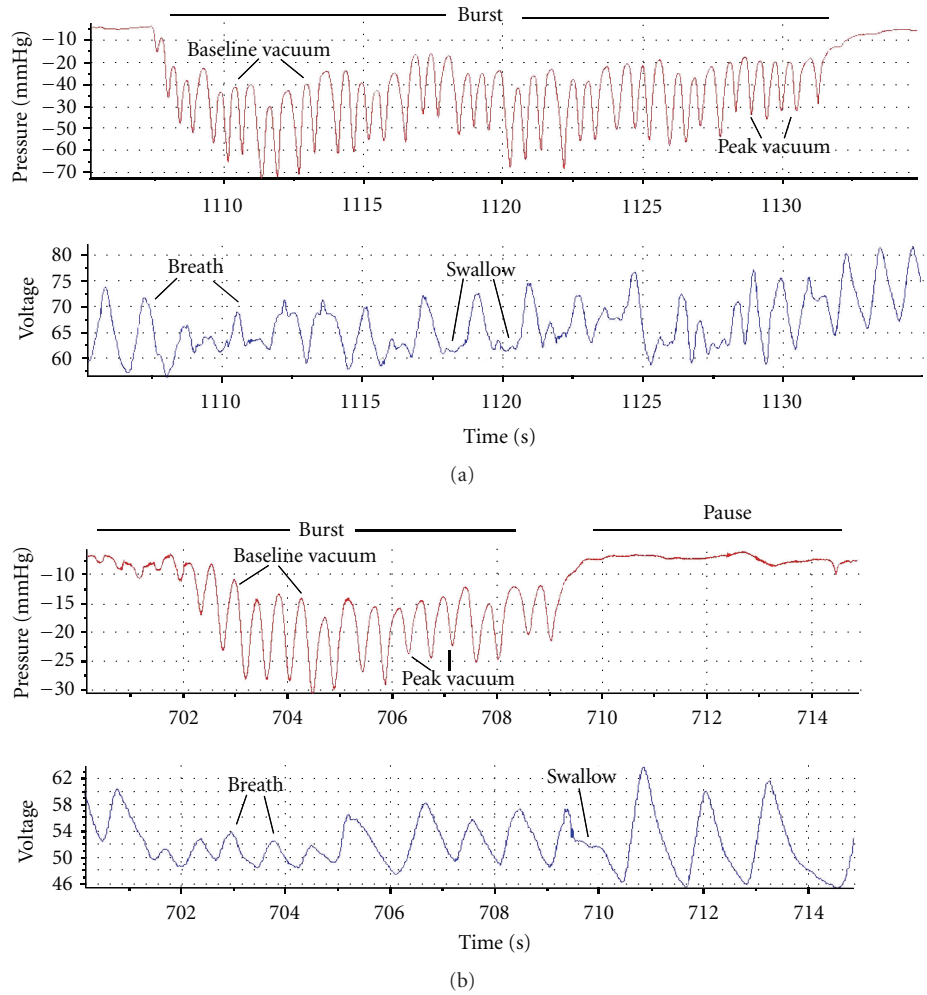

Figure 3: Trace of a suck-swallow-breathe burst of the infant during (a) nutritive sucking (NS) and (b) non-nutritive sucking (NNS) during the monitored feed from the teat. Similar to the breastfeed, during the NS burst, there are more sucks, swallows, and breaths and the burst is longer than NNS.

was higher during NS though the difference between NS and NNS was greater for the breastfeed (interaction; $P = 0.04$), (Table 3).

3.4. Suck, Swallow, and Breathe Ratios. Fewer sucks per swallow were measured during NS than NNS ($P < 0.001$), with no difference in ratios between the breast and teat ($P = 0.14$). No difference was seen in the number of sucks per breath between NS and NNS ($P = 0.08$) or between breast and teat ($P = 0.13$). There were fewer breaths per swallow during NS, and an interaction by feed type ($P = 0.001$) showed, compared to the teat, when breastfeeding that the ratio was higher during NS but lower during NNS (Table 3). Mean SSwB ratios during NS were breast; 3.8 : 1 : 2.2 (range 1 : 1 : 1–12 : 1 : 4) and teat; 3.2 : 1 : 1.9, (range 1 : 1: 1–9 : 1 : 4) and during NNS were breast; 6.7 : 1 : 4.2 (range 2 : 0 : 1–23 : 4 : 23) and teat; 8 : 1 : 5.8 (range 2 : 0 : 2–15 : 1 : 8).

3.5. Burst Characteristics. NS bursts on both the breast (Figure 2) and the teat (Figure 3) were significantly longer than NNS bursts, NP and NNP (all $P < 0.001$), and NS bursts were significantly longer at the breast than on the teat ($P < 0.001$). No differences in duration between the breast and teat were seen between NNS, NP, and NNP (all $P > 0.05$) (Table 4). Across the feed, an interaction ($P = 0.0013$) showed that NS bursts during breastfeeding became shorter as the feed progressed, but this pattern was not seen in teat feeds.

3.6. Sucking and Respiratory Rates . Respiratory rate was not different between the breast and teat ($P = 0.29$). Respiratory rate during NS was slower than during NNS ($P < 0.001$), which was similar to NP ($P = 0.55$) and NNP ($P = 0.81$) rates. Sucking rates were significantly faster during NNS than NS ($P < 0.001$) and did not differ between the breast and teat ($P = 0.61$) (Table 5). Sucking ($P = 0.34$) and respiratory rates ($P = 0.46$) did not change across the feed.

3.7. Vacuum Relationships. Mean vacuum during NS ($P < 0.05$) was stronger than during NNS, which was stronger

Table 3: Number of sucks, swallows, and breaths per burst and the suck : swallow : breathe (SSwB) ratios during the monitored breastfeed and the feed from the experimental teat during nutritive (NS) and non-nutritive (NNS) sucking.

	Burst	Breast	Teat	#*P* value
Sucks	NS	19.5 (12.0, 28.5)	10.5 (7.8, 15.3)	[ii]0.04
	NNS	6.0 (5.0, 9.0)	6.0 (3.8, 9.8)	
	P value*	[ii]0.04		
Swallows	NS	6.0 (3.0, 11.3)	4.0 (2.0, 7.3)	0.38
	NNS	1.0 (0.0)	0.5 (0.0, 1.0)	
	P value*	<0.001		
Breaths	NS	12.5 (7.0, 17.8)	7.0 (4.0, 10.3)	0.7
	NNS	4.0 (3.0, 6.0)	5.0 (2.8, 7.3)	
	P value*	0.03		
Sucks per swallow	NS	3.1 (2.1, 4.9)	2.7 (1.7, 3.7)	0.14
	NNS	6.0 (4.0, 9.0)	6.5 (5.8, 10.0)	
	P value*	<0.001		
Sucks per breath	NS	1.7 (1.3, 2.0)	1.7 (1.2, 2.0)	0.13
	NNS	1.5 (1.0, 2.0)	1.3 (1.0, 1.9)	
	P value*	0.08		
Breaths per swallow	NS	1.9 (1.3, 2.5)	1.6 (1.3, 2.2)	[ii]$P \leq 0.001$
	NNS	4.0 (3.0, 5.0)	5.0 (3.4, 8.3)	
	P value*	[ii]$P \leq 0.001$		

NS: nutritive sucking and NNS: non-nutritive sucking.
Results are median (interquartile range).
*P value between NS and NNS.
#P value between the breast and teat.
[ii]Significant interaction between the breast and teat with NS and NNS.

Table 4: Duration characteristics for the monitored breastfeed and the feed from the experimental teat.

	Burst	Breast	Teat	#*P* value
Sucking duration (s)	NS	8.9 (4.5, 18.3)	5.9 (3.6, 11.7)	0.001
	NNS	4.5 (3.1, 7.1)	2.7 (2.0, 4.9)	0.41
	*P value	0.001	0.001	
Pause duration (s)	NP	3.2 (1.9, 5.7)	2.8 (1.8, 4.5)	0.99
	*P value	0.001	0.001	
	NNP	2.9 (2.2, 4.1)	3.8 (2.2, 6.1)	1.0
	*P value	0.001	0.001	

NS: nutritive sucking, NNS: nonnutritive sucking, NP: nutritive pausing, and NNP: non-nutritive pausing.
Results are median (interquartile range).
*P value compared to NS.
#P value between the breast and teat.

than NP ($P < 0.001$) and NNP ($P < 0.001$) for both breast and teat. All mean vacuum levels were different (all $P < 0.05$), with the exception of NP/NNP, which were similar within the breast ($p = 0.85$) and teat ($P = 0.77$). All mean vacuums were stronger at the breast (all $P < 0.001$) (Table 6).

Stronger peak vacuum was related to a slower suck rate for both the breast and teat, and this effect was greater for the teat (interaction; $P = 0.021$). No relationships were seen between suck rate and mean or baseline vacuum or between respiratory rate and mean, peak or baseline vacuum. Peak vacuum levels during NS were stronger at the beginning of the feed, and this effect was greater in the teat (interaction; $P < 0.001$). No difference was seen for baseline ($P = 0.94$) or mean vacuum ($P = 0.93$) across the feed.

4. Discussion

This study has demonstrated that with the experimental teat, infants are able to maintain oxygen saturation and heart rates similar to those of breastfeeding if vacuum is made the central component of bottle-feeding. In addition, the experimental teat allowed infants to coordinate sucking,

Table 5: Respiratory and sucking rate during the monitored breastfeed and the feed from the experimental teat.

	Burst type	Breast	Teat	#P value
Respiratory rate (breaths/min)	NS	59.2 ± 21.5	55.1 ± 23.9	0.29
	*P value	<0.001		
	NNS	68.1 ± 22.3	68.8 ± 19.5	
	NP	68.7 ± 22.2	66.3 ± 31.8	
	*P value	0.55		
	NNP	70.2 ± 31.3	70.3 ± 31.0	
	*P value	0.81		
Suck rate (sucks/min)	NS	89.1 ± 18.8	88.4 ± 28.1	0.61
	*P value	<0.001		
	NNS	103.9 ± 21.2	105.7 ± 23.4	

NS: nutritive sucking, NNS: non-nutritive sucking, NP: nutritive pausing, and NNP: non-nutritive pausing.
Results are mean ± SD.
*P value compared to NNS.
#P value between the breast and teat.

Table 6: Vacuum relationships during the monitored breastfeed and the feed from the experimental teat.

	Burst type	Breast	Teat	#P value
Mean vacuum (mmHg)	NS	−68.4 (−92.3, −47.2)	−32.4 (−40.5, −25.3)	<0.001
	*P value	0.02	<0.001	
	NNS	−52.9 (−89.9, −31.8)	−21.4 (−29.7, −15.5)	<0.001
	NP	−15.3 (−28.2, −6.7)	−7.9 (−11.3, −4.7)	<0.001
	*P value	<0.001	<0.001	
	NNP	−14.0 (−29.7, −8.3)	−6.9 (−8.0, −5.4)	<0.001
	*P value	<0.001	<0.001	

NS: nutritive sucking, NNS: non-nutritive sucking, NP: nutritive pausing, and NNP: non-nutritive pausing.
Results are median (interquartile range).
*P value compared to NNS.
#P value between the breast and teat.

swallowing and breathing during both NS and NNS in a manner comparable to that used during breastfeeding. These results support recent evidence suggesting that intra-oral vacuum rather than compression is critical to ensure safe and coordinated milk removal from the breast [9].

4.1. Oxygen Saturation and Respiration. In contrast to many other studies [2–5, 23], oxygen saturation, heart rate, and respiratory rates were not different when infants fed from the breast or the experimental teat (Table 2). It is likely that infants were able to control the flow of milk more easily with the experimental teat than traditional teats as no milk would flow when they stopped sucking or if they compressed the teat; however, we have not measured other teats in this study. Traditional teats with high flow rates are associated with reduced oxygen saturation, altered respiratory rate and bradycardia in both term and preterm infants [2–5]. Certainly Goldfield et al. [5] found differences in oxygen saturation between breastfeeding and two different bottle systems, the first a soft-walled bottle and nipple, and the second a hard-walled bottle and nipple. The authors showed that oxygen saturation was significantly higher during breastfeeding than during feeds from the second bottle system only and that feeds from the first system showed higher values than feeds from the second system. Our results and those of others [5] suggest that the design of the bottle/teat influences infant oxygenation and show that the requirement of vacuum in the teat in our study enabled infants to be physiologically stable in a similar manner to the breast. These findings may be relevant to infants that are not physiologically stable when feeding from teats, such as premature infants where oral feeding with a teat often results in desaturation and bradycardic episodes when learning to feed [2, 4, 24, 25].

4.2. Suck, Swallow, and Breathe Coordination. Infant SSwB coordination was not compromised when feeding from the experimental teat. Differences between NS and NNS were similar for both breastfeeding and feeding using the teat. There was a greater difference in the total number of sucks between NS and NNS for the breastfeed compared to the teat (Table 3), which is most likely due to milk only being released from the breast during milk ejection, where milk flow rate increases and decreases rapidly over approximately 90 seconds. On average, there are 2.5 milk ejections in a breastfeed [26], and the infant must take advantage of these periods of increased milk availability in order to

feed efficiently. In contrast, conventional teats often provide continuous milk flow and result in more sucks per burst compared to breastfeeding [13]. Certainly similar S : B ratios for the breast and experimental teat suggest that the infant was able to regulate its sucking and breathing such that it was able to maintain good oxygen saturation (Table 2) whether feeding from the breast or the teat.

SSwB ratios were expected to differ between NS and NNS due to longer suck bursts and more frequent swallowing during milk flow, interestingly we showed this pattern on both the breast and teat. During NS the lower number of sucks per swallow (breast, NS; 3.1 versus NNS; 6.0, teat NS; 2.7 versus NNS; 6.5) and breaths per swallow (breast, NS; 1.9 versus NNS; 4.0, teat NS; 1.6 versus NNS; 5.0) on the breast and teat, was in agreement with Weber et al. [27] who noted that S : Sw ratios [28] increased from 1 : 1 to 2 : 1–3 : 1 later in the feed, suggesting a response to decreasing milk flow. Goldfield et al. [5] showed that during breastfeeding swallows occurred in an organised manner and did not appear to interrupt sucking, whereas during feeds from the second (hard-walled) bottle system, swallowing occurred more frequently and in a random/disorganised manner. The authors suggested these factors most likely contributed to the periods of desaturation occurring on the second system [5]. Again these results demonstrate that bottle design influences the infant's coordination and supports the notion that similar SSwB coordination between the breast and teat in this study is a result of the vacuum requirement for milk removal. It is not clear whether this is because the infant is regulating milk flow at will or that the coordination of tongue action and application of vacuum is also influencing SSwB coordination, but both are likely to be contributing factors.

4.3. Burst Characteristics. Burst duration patterns were similar during breastfeeding and feeding from the experimental teat, though the longer NS bursts observed during breastfeeding may indicate that infants were maximising milk removal during milk ejection. As highlighted earlier, milk ejection is a transient phenomenon lasting approximately 90 seconds [29] and feeding is most efficient if the infant takes advantage of this period of increased milk availability, whereas with a bottle the milk is always available. Despite this, there was no difference in the total time spent NS between the breast and the teat (Table 1). The fact that pause durations were also similar between the breast and teat (Table 4) is consistent with the infants maintaining adequate oxygenation. Certainly preterm infants have shown longer pauses during bottle-feeding than breastfeeding indicating the need to recover from long sucks bursts [2].

The difference in milk transfer between the breast and teat may have been constrained by the volume of milk available in the bottle in that infants might have transferred a greater volume if more milk was available. Despite this, the NS transfer rate between the groups was the same. Contrasting results have been published with conventional teats. Taki et al. [13] showed that during a bottle-feed, despite similar milk transfer, suck bursts were longer and less frequent compared to breastfeeding. Our results show that the requirement of a threshold vacuum for milk removal enabled infants to regulate suck bursts and therefore allows for variation in infant feeding patterns across a feed, whereas other bottles that allow milk flow without sucking may interfere with the infant's ability to regulate suck bursts and alter patterns of breathing and swallowing [2–5].

4.4. Vacuum and Sucking Relationships. When feeding from both the breast and the experimental teat, NNS sucking rates were faster than that of NS, and mean vacuum levels were weaker during NNS, though vacuums were higher at the breast. Stronger breastfeeding vacuums can be explained by rapid changes in rates of milk flow (milk ejection) and the level of vacuum required for milk removal. During breastfeeding, the infant must apply a baseline vacuum to elongate and position the nipple within the oral cavity such that milk removal and swallowing is optimal [14]. The cyclic vacuum applied must then be strong enough to expand the nipple, and may be altered in response to changes in milk flow [14]. Both the threshold vacuum required for milk removal and flow rate of the teat are likely factors that have resulted in lower vacuums being applied by the infant during bottle feeding. Adaption to different feeding conditions has been shown previously, in particular to the rate of milk flow [30, 31]. Despite this, suck rates were not different for the breastfeed and feed from the experimental teat. Previously rates have been shown to differ with different types of teats, where higher flow teats are associated with faster suck rates and low flow teats with slower suck rates [31]. It is possible that the suck rate was similar between the breast and teat because infants were conditioned to sucking on the breast; however, given that previous studies have shown consistently higher suck rates on the bottle than the breast [13], we suspect that suck rates were similar for the vacuum release teat because the infant in fact controlled the rate of milk removal.

We have shown that by removing compression and relying on vacuum only for milk removal oxygen saturation, heart rate, and SSwB patterns were not different between the breast and teat. Previous studies have shown that infants apply an alternating pattern of compression and vacuum to traditional teats, where milk is removed when the tongue is compressing the teat producing positive pressure rather than as the tongue is lowering, creating negative pressure [10, 30, 32]. The experimental teat enabled some compression of the teat in the second half of the suck cycle when milk was cleared from the oral cavity but excluded the possibility of milk removal with compression in the first half of the suck cycle. Although infants were only exposed to the experimental teat once, they were able to produce SSwB patterns and vacuum in a similar manner to breastfeeding suggesting rapid adaptation [14]. The long-term outcomes of feeding from both the experimental teat and breastfeeding were not measured in this study and warrant further research. It is not clear whether the high acceptability of the teat was due to the central vacuum component or the intrinsic capability of the infant in its ability to adapt to different feeding environments, we suspect that both these factors may be important.

5. Conclusion

This study has demonstrated that when the application of vacuum, rather than compression, is required for milk removal from a teat, oxygen saturation, heart rate, and SSwB patterns are not different to those measured during breastfeeding. These results suggest that vacuum may be conducive to safe and coordinated milk removal by the infant during both breast and bottle-feeding.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Research Article

Infant Feeding Practices and Nut Allergy over Time in Australian School Entrant Children

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Aim. To measure the association between infant feeding practices and parent-reported nut allergy in school entrant children. *Method*. The Kindergarten Health Check Questionnaire was delivered to all 110 Australian Capital Territory (ACT) primary schools between 2006 and 2009. Retrospective analyses were undertaken of the data collected from the kindergarten population. *Results*. Of 15142 children a strong allergic reaction to peanuts and other nuts was reported in 487 (3.2%) and 307 (3.9%), children, respectively. There was a positive association between parent reported nut allergy and breast feeding (OR = 1.53; 1.11–2.11) and having a regular general practitioner (GP) (OR = 1.42; 1.05–1.92). A protective effect was found in children who were fed foods other than breast milk in the first six months (OR = 0.71; 0.60–0.84). *Conclusion*. Children were at an increased risk of developing a parent-reported nut allergy if they were breast fed in the first six months of life.

1. Introduction

Peanut sensitisation and allergy in children is increasing both in incidence and prevalence in various parts of the world [1–3] although no studies to date have demonstrated this trend in Australian children.

The evidence of the role of infant feeding practices in protection against, or causation of, peanut allergy is inconclusive. Despite breast feeding being recommended as the sole source of nutrition for the first 6 months of life [4], an increasing number of studies have implicated breast feeding as a cause of the increasing trend in nut allergy [5–7].

The timing of introducing complementary foods, including foods and/or fluids other than breast milk to infants, has changed over the last 50 years. In the 1960s, most infants had been exposed to complementary foods by 4 months of age. By the late 1990s, expert guidelines recommended delayed introduction of complimentary foods such as solids until after 6 months of age [8, 9]. Delayed introduction of complimentary foods has been challenged by recent population studies which suggest that the current practice of delaying complementary foods until after 6 months of age may increase rather than decrease the risk of allergy [10–15].

Understanding how infant feeding practices might influence the risk of children developing nut allergy is of particular importance given that peanut allergy accounts for two-thirds of all fatal-food induced anaphylaxis [16].

This study measures the association between infant feeding practices and peanut (and other nut) allergies in school entrant children in the Australian Capital Territory (ACT) between 2006 and 2009.

2. Method

All new entrants to primary schools with parent-reported nut allergy were selected from those who took part in the ACT Kindergarten Health Check between 2006 and 2009. The Health Check Questionnaire (HCQ) has been described elsewhere [17]. Data are collected on the child's demographics and parents are asked to report on a variety of health issues in their child. Of the 17401 HCQ sent between 2006 and 2009, 15258 (88%) HCQ were completed and parents of 15142 children consented to the data being used for research purposes.

A positive response to the question "Has your child ever had a strong allergic reaction to peanuts/peanut products,

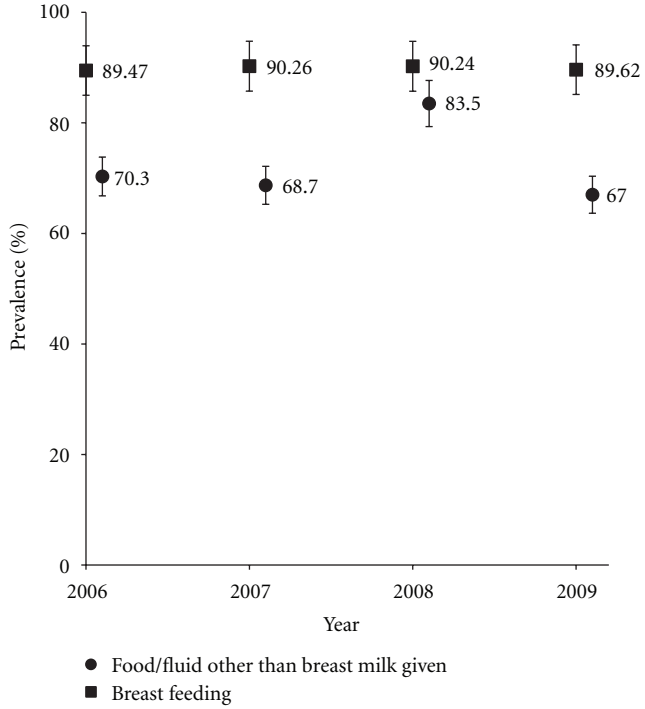

Figure 1: Infant feeding practices between 2006 and 2009.

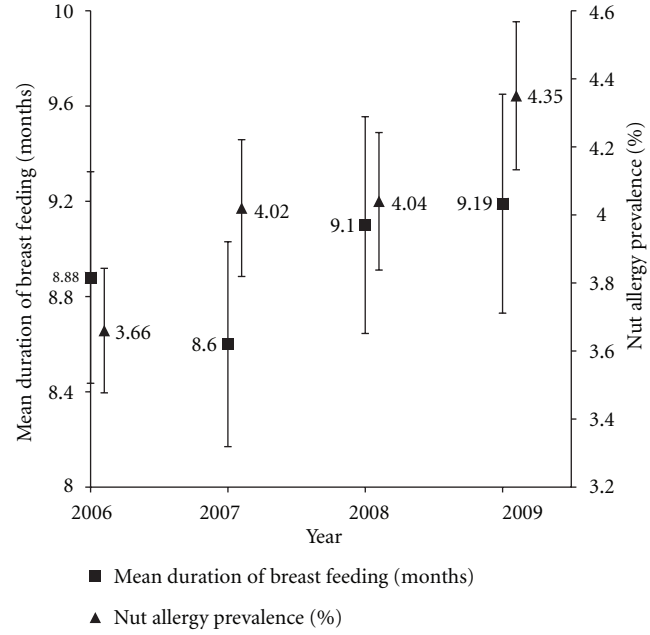

Figure 2: Nut allergy prevalence and mean duration of breast feeding between 2006 and 2009.

and/or other nuts/nut products?" in the HCQ was termed as parent reported nut allergy.

Parents reporting their child was breast fed were asked, "What age was your child when breast feeding ceased?" Parents were also asked: "Was your child fed any fluids or food, other than breast milk for the first 6 months?" and (*parents were asked to tick all that apply*) "Fluids and liquids offered in the first 6 months were: (i) water, (ii) fruit juices, (iii) baby cereal, (iv) vegetables, (v) formula milk, (vi) other..."

The prevalence of peanut and other nut allergy reported in the HCQ was estimated with binomial-based 95% confidence intervals (CIs). Demographic characteristics and infant feeding practices of children with and without reported nut allergies were compared using chi-square tests or regression models with adjustment for age and sex. Clean, nonidentifiable data were analysed using Statistical Package for Social Sciences (SPSS) (version 17.0). The ACT Department of Health Research Ethics Committee approved this study.

3. Results

The HCQ was delivered to all 110 primary schools in the ACT. Parents of 15142 children completed the HCQ and consented to the data being used for research purposes. Parents reported that 487 children had allergy to peanuts and 592 to peanuts and/or other nuts. The overall prevalence of reported nut allergy was estimated as 3.9% (95% CI: 3.6–4.2%) and the prevalence of reported peanut allergy was 3.2% (95% CI: 2.9–3.5%).

13422 (88.6%) children were breast fed (1.4% unknown). The mean duration of breast feeding was 9.9 months (95% CI: 9.82–10.08).

(65.4%) children were fed food and/or fluids other than breast milk in the first six months (2.2% unknown). Of these children, 46.3% were given baby cereal, 46.4% formula milk, 33.5% vegetables, 13.5% fruit juices, and 13.7% other foods. Children who were exclusively breast fed were more likely to have a nut allergy (OR = 1.43; 1.21–1.69; $P = 0.000$), whilst nut allergy was less likely to occur in children only fed food and/or fluid other than breast milk (OR = 0.63; 0.45–0.89; $P = 0.009$) and those who were both breast fed and given other food and/or fluid (OR = 0.83; 0.70–0.98; $P = 0.025$).

Comparative data on infant feeding practices and nut allergy are presented in Table 1.

Children with nut allergy were more likely to have been breast fed (OR = 1.53; 1.05–1.92; $P = 0.010$) and have a regular GP (OR = 1.42; 1.05–1.92; $P = 0.023$) than those with no allergy. Protection against nut allergy was found in children who were fed food and/or fluids other than breast milk before six months (OR = 0.71; 0.60–0.84; $P = 0.000$) (Table 2).

Infant feeding practices remained relatively unchanged between 2006 and 2009. Figure 1 compares the proportion of children breast fed with the proportion of children fed food and/or fluids other than breast milk in the first six months. Figure 2 compares the prevalence of parent-reported nut allergy and the mean duration of breast feeding between 2006 and 2009.

4. Discussion

Our study suggests nut allergy prevalence in school entrant children is increasing in the ACT. The finding is consistent

Table 1: Odds ratios for reported nut allergy and infant feeding practices of kindergarten children in the Health Check Questionnaire 2006–2009.

Infant feeding practice in the first 6 months	Reported nut allergy*			Odds ratio (95% CI)#	*P* value
	Yes		No		
Breast feeding only ($n = 4647$)	232	(5.0%)	4415	1.43 (1.21–1.69)	0.000
Other food/fluid only ($n = 1312$)	35	(2.7%)	1277	0.63 (0.45–0.89)	0.009
Breast feeding + other food/fluid ($n = 8368$)	311	(3.7%)	8057	0.83 (0.70–0.98)	0.025
Unknown ($n = 138$)	4	(2.9%)	134		

* Parental response to the question: "Has your child ever had a strong allergic reaction to peanuts/peanut products, and/or other nuts/nut products?"
#Adjusted for age and sex where appropriate. Children with invalid data on the nut allergy and feeding practice questions were excluded from comparative analyses.

Table 2: Odds ratios for reported nut allergy and characteristics of kindergarten children in the Health Check Questionnaire 2006–2009.

Characteristic of child		Reported nut allergy*		Odds ratio (95% CI)#	*P* value
		No	Yes		
Sex of child	Male	7184	322	1.15 (0.97–1.35)	0.105
Age	5 years	12257	511	1.11 (0.87–1.42)	0.385
Aboriginal or Torres Strait Islander	Yes	238	11	1.11 (0.60–2.05)	0.737
	Unknown	417	19		
Child has a usual general practitioner	Yes	12503	543	1.42 (1.05–1.92)	0.023
	Unknown	92	2		
Child was breast fed	Yes	12599	549	1.53 (1.11–2.11)	0.010
	Unknown	101	2		
Duration of breast feeding (mean 9.9 months)	—	—	—	1.02 (1.01–1.03)	0.000
Child was fed food or fluids other than breast milk in first 6 months+	Yes	9371	347	0.71 (0.60–0.84)	0.000
	Unknown	197	9		

* Parental response to the question: "Has your child ever had a strong allergic reaction to peanuts/peanut products, and/or other nuts/nut products?"
#Adjusted for age and sex where appropriate.
+Includes children who were breast fed and given other food/fluid, and those who were exclusively given other food/fluid.

with current evidence depicting Australia as part of a global trend of increasing nut allergy [1–3]. The prevalence of nut allergy for five-year-old children in the ACT is 3.9%, with peanut allergy accounting for 3.2%, which is almost twice the prevalence of British school entrant children (1.8%) [18].

Australian guidelines do not recommend avoiding foods in pregnancy or lactation for preventing allergic disease in infants [19]. These recommendations are made on the best available evidence at the time of writing, however, emerging evidence has proven that maternal diet during lactation is a route of allergen exposure which may result in sensitisation [20–23]. In several studies, maternal ingestion of peanut during pregnancy or lactation was shown to increase the risk of peanut allergy [21, 22]. Furthermore, in a case study of an allergic reaction to peanut in an exclusively breast fed two-week-old boy, it was concluded that the clinical symptoms of allergy could only be explained by occult ingestion through breast milk rather than environmental exposure [5]. In a study of 122 children with nut allergy, it was noted that 83% of these children were breast fed and that over 90% of the mothers admitted to ingesting peanut during lactation [24].

Our study found that almost 90% of children were reported to have been breast fed as infants and that over 4% of these children were reported to have a nut allergy. Of the remaining 10% of children who were reported not breast fed, parent-reported nut allergy prevalence was 2.72%—the likelihood of developing a reported nut allergy was 1.5 times higher in breast fed children than in nonbreast fed children. These findings are replicated in earlier studies which also concluded that nut allergy was more likely in children who had been breast fed [25].

These results contribute to the argument that breast feeding by itself does not appear to be protective against nut allergy in children, and that it may in fact be causative of allergy [5–7].

We observed an increasing trend in prevalence of parental-reported nut allergy between 2006 and 2009 confirming the findings of previous studies [1, 2]. This may be a reflection of increased consumption of peanut and peanut products by pregnant and nursing mothers [26]. It has also been argued that peanut sensitisation may occur via other subtle environmental routes, such as the application of peanut oil containing lotions to the child or the breast feeding mother [27], however, this argument is controversial [28].

Other infant feeding habits were also analysed in this study. We found that children fed only foods other than breast milk before six months were least likely to develop a parent reported nut allergy (OR = 0.63; $P = 0.009$)

compared with children who were exclusively breast fed (OR = 1.43; $P = 0.000$). Children who were both breast fed and given other foods and/or fluids before six months were also protected (OR = 0.83; $P = 0.025$). The minimisation of the occult exposure of allergen that may occur with breast milk and the unknown maternal ingestion of peanut products may explain our result [5, 20]. Our findings are consistent with several studies which concluded that early introduction (prior to 4 months age) of complementary food was associated with a reduced risk of peanut sensitisation [29] and eczema in children with allergic parents [30] and that a more diverse diet of solids before 4 months was associated with a lower risk of sensitization at 6 years [15].

Furthermore, prolonged breast feeding has been shown to increase the odds of developing peanut allergy by almost 3 times that of children who were weaned at or before 6 months [27]. Our study results concur, finding a parallel between longer breast feeding time and an upward trend in parental reported nut allergy.

Our study results also confirmed our previous finding of a positive association between parental reported nut allergy and the child having a regular GP [25]. We speculate that children with an allergy are more likely to see a regular GP for diagnosis, follow-up, and prescription for adrenalin if the allergy is severe.

Limitations in this study include inconsistency in interpreting the words "strong reaction" in the HCQ. Parents may have interpreted the words "strong reaction" in the screening question to mean either a reaction to a diagnostic test or a clinical reaction. Also, the study design does not allow causality to be inferred. Finally, data were collected four or five years after infant feeding habits had ceased—accuracy of recall impacts upon results. A strength of this study is that the data describe the association between breast feeding and parent reported nut allergy in a sample population that is highly representative of the ACT community of school entrant children. The large sample size, number of years of data collection and high response rate has reduced the chance of random error. However, there is the possibility of over-representation of a Caucasian population in the ACT and hence extrapolation of these findings onto ethnic populations is not appropriate.

5. Conclusion

Children who were breast fed in the first six months of life were at an increased risk of developing a parent-reported nut allergy. A protective effect against parent-reported nut allergy was found in children who were given food/fluid other than breast milk before six months either exclusively or in combination with breast milk. There was a positive association between parent-reported nut allergy and having a regular GP. With the rate of parent-reported nut allergy in a highly representative sample population being 1 in 25 and peanut allergy being 1 in 30, there is a need for further research in this field. The scarce and often contradicting evidence regarding nut sensitisation and infant feeding practices demands particular emphasis.

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Review Article

It Takes a Mouth to Eat and a Nose to Breathe: Abnormal Oral Respiration Affects Neonates' Oral Competence and Systemic Adaptation

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Mammalian, including human, neonates are considered to be obligate nose breathers. When constrained to breathe through their mouth in response to obstructed or closed nasal passages, the effects are pervasive and profound, and sometimes last into adulthood. The present paper briefly surveys neonates' and infants' responses to this atypical mobilisation of the mouth for breathing and focuses on comparisons between human newborns and infants and the neonatal rat model. We present the effects of forced oral breathing on neonatal rats induced by experimental nasal obstruction. We assessed the multilevel consequences on physiological, structural, and behavioural variables, both during and after the obstruction episode. The effects of the compensatory mobilisation of oral resources for breathing are discussed in the light of the adaptive development of oromotor functions.

1. Introduction

During the first months of life, mammalian infants are considered to be "obligate nose breathers" [1], although the qualifying term "preferred nose breathers" was proposed subsequently [2]. In any event, these wordings highlight the fact that newborn and very young mammals depend on nasal breathing to adapt their behaviour competently, especially in relation to ingestion and, in newborns, to sucking—the specialisation of oral behaviour evolved by mammalian infants in response to mothers having evolved nipples or teats as appendages for milk transfer [3].

While the motor process underlying respiration relies on a centrally controlled automatism, its execution has multiple general consequences, beginning with the most peripheral structures that channel airflow. The resistance of air through the nasal passages has a formative effect on the nasal cavities [4]. Under normal breathing conditions, alternating conchae as well as the nasal cycle within the nose lessens the airflow speed and creates turbulent flow conditions that contribute to shape nasal structures. The nasal inflow also "acclimatises" the physicochemical properties (temperature, hygrometry, and cleanliness through dust adsorption) of incoming air, thus optimising both pulmonary exchanges and chemosensory reception [4–6]. The upper airway crosses the oral path in the larynx region, where respiration and ingestion (and sometimes egestion) are rendered exclusive by the epiglottic switch during feeding. In newborn and suckling infant mammals, both pathways are mobilised serially during ingestive sequences as breathing is compatible with sucking (and later mastication) but not with swallowing [7–10]. Therefore, hazardous aspirations into the lower respiratory pathway are in principle avoided while sucking [11], except during feeding in preterm infants [12]

Beyond ingestion, nose breathing is the dynamic component of olfaction, either in its baseline form through regular inhalation/exhalation or in a specific form characterised by an accelerated rhythm or deeper inhalations, called sniffing. Both inhalation forms create an air flow that carries volatile compounds to intranasal chemosensory nerve endings that give rise to olfaction and trigeminal sensations. Corresponding sensory inputs promote guidance to the offspring to

reach the breast, to encode milk/food odour as rewarding (retronasal olfaction), and to monitor the caregiver's odour (orthonasal olfaction) for identity recognition, solace, and energy conservation [13]. Finally, nasal trigeminal sensation caused by the incoming airflow constitutes a regulatory input to the respiratory rhythm generator [14], and its silencing through nasal occlusion may thus subsequently alter respiratory performance.

Nasal breathing is thus multiply related to adaptive oral function, that is, by ensuring an ongoing supply of oxygen during food intake and by supporting olfaction and related motivation to seek the food source and sustain feeding. Thus, any disruption of the normal nasal breathing route through (uni- or bilateral) nasal obstruction may affect not only the respiratory function but also all interdependent sensorimotor nasal, oral, and laryngeal functions. Nasal obstruction can result from either congenital or postnatal causes and may amplify resistance to air-flow and impair sucking-swallowing responses, with increased risks of aspiration or of more severe and threatening respiratory distress conditions [15]. In addition, nasal obstruction alters the "trophic" flow of sensory information towards the olfactory brain. Sensory deprivation due to early nasal obstruction has indeed repeatedly been demonstrated to alter both the structure of animals' olfactory tracts and related functions [16–18].

Here, we present and discuss the immediate and deferred effects of constraining neonatal organisms to breathe through the mouth in response to obstructed or closed nasal passages. A brief survey of human newborns' and young infants' responses to nasal obstruction is paralleled with the results obtained by an experimental approach using neonatal rats. Nasal obstruction was induced experimentally in newborn pups to assess the multilevel consequences on physiological, structural, and behavioural variables, both during and after the obstructive procedure. The effects of respiratory impairment are discussed in light of adaptive development of oromotor functions.

2. Impact of Nasal Obstruction in Human Neonates and Infants

2.1. Causes of Nasal Obstruction. Natural causes of complete nasal obstruction are rare, but vary in human newborns and infants. The most extreme forms are due to congenital laryngomalacia, bilateral choanal atresia, or oronasal defects associated with Pierre Robin syndrome [19]. Less extreme forms involve choanal stenosis, unilateral choanal atresia, or defects of the nasal septum related to cleft palate [20]. Other mechanical causes such as those due to obstructive tissue masses (adenoid or/and tonsillar hypertrophy) prevail during later development. More benign, short-term obstructive forms derive from mucosal accumulation due to neonatal infections or allergic rhinitis [21–23]. Rhinitis symptoms result from dilation of venous capacitance vessels in the nasal mucosa, mucosal edema, and excess secretions. Allergic rhinitis is very common in infants and children [40% of children are affected in United States; e.g., [24]], as is adenoidal and tonsillar hypertrophy.

Finally, iatrogenic interventions relying on nasogastric tubes or narial tape also have an effect on nasal patency [23]. Inserting nasogastric feeding tubes produces an important (unilateral) increase in nasal airway resistance, thus leading to an increase in respiratory effort [25, 26].

All the above-mentioned causes of obstructive nasal airways can be associated with physiological conditions that may potentiate their effects. First, nasal resistance is greatest during infancy, when airways are narrower [27]. Thus, we can expect that the effects of nasal obstruction would be more important for small-sized bodies, namely, newborns, and even more preterm newborns. Second, nasal patency fluctuates normally between the two nasal cavities by changes in the engorgement of the mucosal vessels in the middle and lower turbinates, a normal variation known as the "nasal cycle." The magnitude of nasal resistance alternates in each nasal cavity every 2 to 4 h in 60–70% of healthy individuals [28]. Finally, posture can substantially influence the degree of vascular congestion in the nose. Nasal obstruction increases bilaterally as a subject assumes the supine position and increases in the dependent nasal passage in the lateral recumbent position [29].

2.2. Short- and Long-Term Impacts on Oral Function. Nasal obstruction forces normal nasal breathing into oral breathing. Numerous clinical observations and experiments show that this apparently benign change has in fact immediate and/or deferred cascading effects on multiple physiological and behavioural functions. First, it has an obvious perturbing impact on newborns' and very young infants' sucking-swallowing activities, and growth is affected accordingly [20]. Nasal obstruction in older infants and children, linked to hypertrophied adenoids or tonsils, is related to growth stagnation, which normalises after surgery [30–32]. It also affects young infants' behaviour, for instance increasing crying episodes and sleep perturbed by more apneic spells, and can be involved in the sudden infant death syndrome [26, 33, 34].

Nose blocking also affects nasal chemosensation [35]. The disturbing effects of nasal closure during early development on the olfactory tract and function have been extensively demonstrated (mainly in the rat; e.g., [16]). Evidence for early structural alterations of the sense of smell due to nose-blocking is less well documented for humans [35, for review], but evidence shows that children's olfactory performance is significantly reduced [36, 37]. The clearest effect of adenoid-related nose blocking on olfaction is evidenced by the postoperative recuperation of children's awareness of food odours and their subsequent appreciation of eating [36]. Similar perceptual effects may operate in younger, preverbal infants when their nasal respiration and, hence, olfactory abilities are temporarily suppressed and then resume.

More or less chronic oral breathing has repeatedly been shown to induce a prolonged imbalance of orofaciopharyngeal muscle activity. According to Moss [38], the muscular activity related to nasal breathing allows proper development of the craniofacial complex interacting with other functions such as mastication and swallowing [39]. This theory is based on the principle that facial

growth depends on the functional activity of the different components of the head and neck region. For example, oral breathing imposed by adenoid hypertrophy has been suggested to explain the posterior rotation of the mandible [40]. Thus, oral breathing has been associated with increased mandibular inclination and changes in normal facial proportions, characterised by increased anterior lower facial height and decreased posterior facial height [41–43]. This induces the vertical axis of the facial skeleton to tend to develop excessively, resulting in an ogival palate (with consequences on dental occlusion) and dolichocephaly (or "long face syndrome"; [44, 45]) Similarly, experimentally induced nasal obstruction in young macaques (before and during pubertal development) induced permanent craniofacial deformities [46]. Long-term consequences of this developmental plasticity as a function of oral-breathing-induced craniofacial muscle mobilisation appear to be gender specific. For example, preschool boys suffering respiratory disorders during sleep presented higher anterior lower facial heights than girls [47].

To sum up, the shift from typical nose breathing to atypical mouth breathing in neonates and young infants illustrates how one function can have cascading effects on other functions to finally affect future form and functions. This forced change to oral respiration may impact all functions, from the most local [e.g., muscular exertion, craniofacial growth and functioning, chemosensory awareness, eating (sucking-swallowing articulation), and lower airway development] to the more general [sleep quality, temperamental traits, stress reactivity, and quality of life].

3. Multiple Impacts of Nasal Obstruction: The Rat as a Model

3.1. The Model: Methods and Outcome Measures. To further our understanding of nasal obstruction effects in general, we decided to investigate this problem in detail by using an animal model. For many reasons we chose the rat. To assess how momentarily perturbed nose breathing can affect oral competence as well as more general behavioural and physiological functions, an experimental technique of reversible bilateral nasal obstruction was developed that could be applied to newborn rats during their second week of life. After a first week of normal development, the pups underwent nasal closure for about 5 days to mimic the outbreak of a short blocking of nasal patency during early development of organic structures and functions. Nasal obstruction was performed on postnatal day (PND) 8 by bilaterally closing the external nares using an anaesthetic/surgical procedure currently applied to investigate the effects of closed nostrils on emerging olfactory function [48–51]. This procedure induced complete nasal closure between PND 8 and 12, with progressive reversal to unrestricted nasal airflow after PND 14. Different variables were measured on PND 9, that is, 24 h after the closure of both nostrils, on PND 15 to evaluate immediate and short-term effects, and up to PND 90 to evaluate long-term effects. The closed-nose (CN) pups were compared to sham-operated open-nose (ON) pups and to control (C) pups to evaluate oral competence and performance.

The following variables were quantified to assess the impact on organismal functioning, from the more local to the more general consequences: *feeding behaviour* [sucking behaviour of individual pups (nipple grasping ability, gastric content); maternal responses to pups (pup retrieval, presence in nest, and licking pups)]; *feeding-related structuresand functions* [oral activity; weight and myosin content of orofacial muscles; craniometric parameters]; *olfaction* [olfactory bulb size; nipple grasping performance; discrimination ability]; *metabolism-related consequences* [glycaemia, osmolality, hydration, and growth parameters]; *stress-related consequences* [weight of adrenal glands, plasmatic level of corticosterone, testosterone, and thyroid hormones].

3.2. Oral Competence: Functions—Oral Activation, Food Intake, and Feeding Interactions. Immediately after nasal blocking, the pups' inspiratory activity was redirected through the mouth as inferred from mouth-opening responses. This effect peaked on PND 11 ($n = 23$ mouth openings/min) to regress ($n = 15$ mouth openings/min) when nasal inspiration resumed on PND 14-15 [52]. In the same time, ON and C pups never exceeded 2 mouth openings/min. The fact that the respiratory effort is reassigned to the mouth may interfere with oral competence during suckling. Rat pups' oral performance was assessed directly by their capacity to grasp nipples orally after a period of separation from their dam, and by sucking success, directly evaluated by gastric milk content after a suckling trial. Significantly fewer CN pups than ON and C pups were able to attach to nipples between PND 9 and 15 [53]. In addition, during the days of enforced oral breathing, the sucking efficiency of CN pups that could suck was lower than that of ON and C pups, as shown by the significantly lower amounts of milk in their stomachs [54, 55]. Thus, nasal obstruction clearly interferes with normal sucking performance. First, pups appeared less proficient in attaching to nipples. Second, pups that did attach to nipples extracted milk less efficiently. When the nares had reopened by PND 15, the relative weights of milk taken became similar between groups for female pups, but were higher for CN male pups than for ON and C male pups [53]. So the impact of enforced oral breathing on pup feeding behaviour appeared to be restricted to the period of nose closure, but males expressed compensatory effects and ingested more milk when nasal respiration had been recuperated.

3.3. Oral Competence: Musculo- and Craniofacial Structures and Functions. The redirection, under experimental conditions, of newborn rats' breathing flow from the nose to the mouth recruits all reactive resources to ensure sufficient responsiveness of the organism. The new developmental situation imposed by blocking the nose alters the typical physiological constraints on local muscles and changes the mechanical stress on local bones. Muscles normally mobilised to fulfil respiration then incur extra work to keep the homeostasis of blood gases, but muscles involved

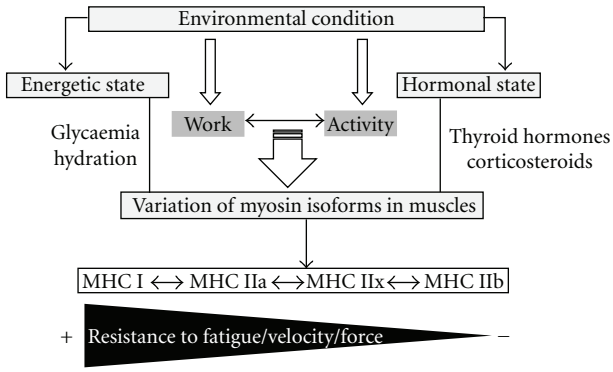

Figure 1: Diagram presenting the impact of environmental condition on myosin heavy chain (MHC) expression in adult skeletal muscles (I: slow; IIa: fast; IIx: fast; IIb: fast type fibres).

in sucking and in social interactions are also recruited to maintain a satisfactory respiratory level.

Skeletal muscles are composed of a combination of fibres classified on the basis of their contraction speeds and resistance to fatigue due to iterative stimulation, as slow twitch or fast twitch [55]. The contractile properties of muscles correlate with their myosin heavy chain (MHC) composition [56–58]. *Adult* skeletal muscles contain four major MHC isoforms, three being of the fast type (MHC IIa, IIx, and IIb) and one of the slow type (MHC I) [59]. MHC isoform expression determines muscle fibre contractile properties: fibres expressing MHC I generate less maximum specific force, slower shortening velocity, and greater resistance to fatigue than fibres expressing fast MHC isoforms (and among fast fibres, those expressing MHC IIx and IIb generate greater maximum specific force, faster shortening velocity, and lower resistance to fatigue than fibres expressing MHC IIa). Some MHC isoforms are specific to the perinatal period [60]: embryonic MHC (MHC_{em}) and neonatal MHC (MHC_{neo}). Expression of the different myosin isoforms in skeletal muscles is developmentally regulated [61]. In fast-contracting rat muscles, MHC_{neo} replaces MHC_{em} to become the predominant type by 7–11 days after birth; subsequently MHC_{neo} is replaced by the fast adult isoforms [62–65]. Slow muscle fibres can develop through several pathways, but involve similar myosin isozyme transitions [63, 66]. The quality and quantity of expressed MHC isoforms of skeletal muscles are exceedingly plastic, and their fibre-type profiles can change in response to numerous factors, such as developmental stage, neuromuscular activity, physical mobilisation, and endocrine conditions [67–71]. These functional interactions are summarised in Figure 1.

The nasal obstruction episode in the present experimental series caused early changes in the structural/functional properties of rat pups' respiratory/orofacial muscles. Four muscles were targeted: the diaphragm, the *digastric anterior* (mandible depressor, opening the mouth), the *masseter superficialis* (mandible propulsor, closing the mouth), and the *levator nasolabialis* (involved in nasal flaring and sniffing). First, the relative weights of the last three muscles were considerably reduced (by 35, 33, and 66%, resp.) in pups following nasal obstruction [50, 72]. Further, during nasal obstruction, maturation of these muscles was enhanced in CN pups compared to ON and C pups. This is attested by the inversely correlated decrease of MHC_{neo} and increase of mature MHC isoforms in the diaphragm and orofacial muscles. This effect of oral inhalation was extremely rapid as the muscular differences among treatment groups could be seen within 24 h after obstruction.

During typical development, muscular MHC composition changes in an orderly fashion from embryonic to neonatal to adult fast/slow isoforms [67], and this change appears regulated in time (between 7–11 days after birth). Then MHC_{neo} decreases, disappearing entirely by PND 28 [68]. The short episode of nasal obstruction enforced here (between PND 9 and 11) clearly influenced these developmental changes, as the MHC_{neo} isoform increased normally in ON and C pups (Table 1), but not in CN pups [72]. Thus, nasal obstruction postponed maturational progression of the oral muscles that were recruited to work in respiration.

The early episode of nasal obstruction had *long-lasting effects* on the properties of the muscles considered in the facial-oral sphere, as these effects could be noted on PND 21 [50] and even on PND 90 [72]. The diaphragm of male rats undergoing CN treatment contained more of the MHC I (slow) isoform, and the target orofacial muscles contained more of the MHC IIa isoform at the expense of IIx and IIb isoforms (the most "fatigable"). The orofacial muscles involved in breathing showed an opposite profile, with decreased and increased expression of the MHC IIx isoform in the muscles involved, respectively, in closing and opening the mouth. Thus, the MHC phenotypes of rat pups exposed to a short episode of enforced oral breathing present plastic changes that appeared adaptive following the abrupt transition from nasal to oral breathing. Furthermore, following temporary forced nasal obstruction, the diaphragm and active sniffing muscles appeared consistently more resistant to fatigue in terms of MHC composition [72]. These phenotypic profile changes of MHC composition in CN rats' active sniffing muscles could be explained by decreased flaring and sniffing. The CN rat pups' mandibular muscle controlling mouth opening became more resistant to fatigue than the muscle controlling oral closing. Thus, although this result is explainable in terms of different controls of mouth opening versus closing muscles, temporarily forced oral breathing might produce long-lasting motor modifications in sucking behaviour associated with alterations of respiratory muscles' specific electromyographic activity.

Oral breathing in rat pups also caused long-term changes in craniofacial development. CN pups presented a symmetrical decrease of the vertical development of the nasomaxillary complex and of the longitudinal development of the skull-base [73]. Thus, an early nasal obstruction period was associated with delayed craniofacial development in both male and female pups. However, in the long run (namely, 90 days after nostril reopening), the craniofacial growth delay noted during the period of nasal obstruction did not persist in CN males in which the nasomaxillary complex and skull-base longitudinal axis has been reduced [73]. By contrast, only the longitudinal skull base of CN female pups remained

Table 1: Distribution of myosin heavy chain (MHC) isoforms in selected oral (*digastric, masseter*) and nasal (*levator*) muscles in rats exposed to an early episode of forced oral breathing (CN group) and in control rats [74]. The different MHC isoforms were characterized on PND 11 and 90 (for key to the functions of the different MHC isoforms, see the text). Short-term nasal obstruction, that is forced oral breathing, leads to long-term orofacial muscle fibre adaptation. We observed increases in MHC neonatal and adult type I isoforms in muscles involved with oral breathing, *digastric,* and *masseter*, in CN group versus control on PND11. No changes were observed in the *levator* muscle involved with nasal breathing on PND 11. There are increases in MHC adult type IIb isoforms in muscle involved with oral breathing, *masseter*, and in muscle involved with nasal breathing, *levator*, in CN group versus control on PND 90. Values are given as percentages of total MHC and comparisons were then made using *t*-test with the Bonferroni correction.

MHC isoforms	emb	neo	I	IIa	IIx	IIb
On PND 11						
CN group						
Digastric	7	78*	15*	—	—	—
Masseter	9*	91*	—	—	—	—
Levator	14	86	—	—	—	—
Control group						
Digastric	6	85	9	—	—	—
Masseter	13	87	—	—	—	—
Levator	14	86	—	—	—	—
On PND 90						
CN group						
Digastric	—	—	—	20*	44*	37
Masseter	—	—	—	—	42*	58*
Levator	—	—	—	18*	25*	57*
Control group						
Digastric	—	—	—	24	37	38
Masseter	—	—	—	—	48	52
Levator	—	—	—	1	31	68

*Significantly different from control group at $t = -10.37$ to 26.03, $P < 0.03$ to < 0.001.

somewhat shorter than that of controls as the animals grew older. Thus, the long-term osteologic effects of an early episode of oral breathing vary in relation to pups' sex.

3.4. Nasal Chemosensory Competence: Structure and Function. Nasal obstruction had a significant atrophic effect on the olfactory bulbs; bulbar weight of CN pups was about 30% less than that of control pups at PND 11 [73], and 50% less at PND 21 [50]. This bulbar reduction is relatable to decreased olfactory function as measured directly and indirectly. A test of odour-guided nipple attachment after a 2 h period of mother-offspring separation showed a perturbed response by CN pups (relative to controls) during the narial closure period (PND 9) and immediately after (PND 15), and the success in getting milk (gastric content) was accordingly reduced during the perturbation of olfaction [53, 54]. Further, in a paired choice-test comparing the odours of nest-sawdust and of clean sawdust, latency to choose was longer and duration of orientation towards the familiar nest odour was shorter for 9-day old CN pups than for control pups. By PND 15, when nasal respiration resumed, this difference was reduced due mainly to the return of nasal respiration in female CN pups [53]. Atrophy of the olfactory bulbs persisted in the long term (PND 90) in both sexes [73], although their exploratory and sniffing behaviours in a new environment became normal [74]. However, olfaction appeared to be permanently affected, as adult CN males exhibited impaired responses to sex-related odour cues [74].

3.5. General Systemic Responses (Viability, Homeostasis, Stress, and Behaviour). Early exposure to an episode of nasal obstruction impacts on pup viability. Under our experimental conditions, mortality was nil in both control groups but reached 23% 72 h after narial closure in the CN group. On PND 21, the cumulative death rate reached 37% [52], suggesting that the consequences of perturbed oronasal function are protracted after the episode of nasal obstruction *per se*. This increased mortality rate is certainly multifactorial as all systemic regulations are concurrently affected by the respiratory mobilisation of the mouth. The *first* cause to be invoked is energetic depletion of the NC pups that were less competent in getting milk. *Second*, another immediate consequence of mouth breathing is air swallowing, especially during the process of sucking. Excess gas in the gastrointestinal tract has been noted after nasal obstruction and related to the advent of necrosis and haemorrhages in the gut [75], in addition to diaphragmatic compression and paralysis of ileus leading to the arrest of intestinal transit [76] and increased risk of lethal perforation [77, 78]. A *third* cause involves the respiratory process itself. The effects of imposed oral breathing obviously affect blood gas parameters, leading to acute hypoxia, hypercapnia, and acidemia [79, 80], especially in neonates [81]. Adult rats' blood pH and O_2

partial pressure are reduced 72 h after narial occlusion [82], leading to adverse changes in the homeostasis of blood gases. Nasal obstruction is also associated with an initial decrease in lung growth (PND 9–11), followed by recovery by PND 90 [74]. *Fourth*, NC rat pups' lessened oral competence caused by oral breathing may explain the small, but significant, decrease in plasma glycaemia on the first day of treatment, relatable to the reduced intake of milk reported above. *Fifth*, oral respiration increases evaporative loss, constituting an additional cause of body weight deficit and stress [83]. The significant increases in vasopressin release and plasma osmolality are indeed indicative of dehydration in CN pups [54]. Thus, any event enforcing oral breathing entails whole body dehydration [84]. *Sixth*, homeostasis is further imbalanced because of food-mediated maintenance of neonatal hormonal state. Thus, a few hours deprivation of mother's milk correlates with a significant reduction in thyroxin and an increase of plasma corticosterone levels [51, 53, 72, 85, 86]. Thyroid, renal, adrenal, and gonadal hormones play a key role in early development. An early deficiency in thyroid hormones disturbs brain development (specifically the olfactory system [87]) and delays the maturation of muscles (especially orofacial muscles) [88–90]. Vasopressin and corticotrophin-releasing hormone (CRH) both play a synergistic role in stimulating the release of adrenocorticotropic hormone (ACTH) [90], so vasopressin could possibly enhance the CRH effect during the first days of nasal obstruction-induced oral breathing. This "stress" reactivity might mediate response to nose-blocking surgery and/or dehydration induced by oral breathing [54]. The stress response induced by narial obstruction in 8-day-old rat pups is also evidenced by the hypertrophy of adrenal glands 72 h after treatment [72]. Adrenal hypertrophy is more marked in females (+68% in CN females and +29% in CN males, compared to controls) on PND 21 [51]. These effects did not persist over the long term (PND 90). An increase in plasma testosterone was observed during the nasal obstruction episode and on PND 90 [73]. This suggests that nasal obstruction *via* the olfactory bulb influences gonadotropin secretion that might be mediated by altering gonadal steroid feedback. *Seventh*, nose blocking affects the immune system by suppressing the proliferation of B-lymphocyte precursors [51]. Thymus weight was reduced only in CN females. The thymus is particularly sensitive to stress-associated glucocorticoids, which induce thymocyte apoptosis. *Eighth*, although not documented by our own experiments, nasal obstruction has far reaching consequences on biological rhythms. It can impair nocturnal sleep and induce diurnal lethargy [91–93]. We cannot exclude that it also induced biorhythmic maladaptation in rat pups, in terms either of hyporeactivity when they had to suck the nursing dam or of hyperactivity due to high corticosterone levels. *Finally*, a brief period of nasal obstruction affects mother-offspring interactions and decreases offspring's food intake [53]. Young rats' narial obstruction alters mother-pup interactions by reducing duration of retrieving and increasing pup licking by the dam. As already mentioned above, CN pups also showed lower mean duration of nursing and nipple attachment, which appeared related to difficulties in finding the nipple.

3.6. Summary and Limits of the Model. The abrupt irruption of abnormal conditions of breathing in preweaning rat pups affects many local and general phenotypic traits over both short- and long-term developmental time scales. Oro-naso-facial growing structures and maturing functions are indeed shaped by the way they are solicited by their motor engagement in early respiration and ingestion. Thus, the oral and nasal pathways are tightly interdependent to ensure continued breathing when nasal occlusion occurs. However, this nasal defect-related oral compensation has immediate, short-term and long-term consequences (Figure 2).

The experimental results using the neonatal rat model of nasal occlusion may not be extrapolated in full to infants of other species. Thus, total obstruction of human infants' nasal airflow as in our model may be rare, as it is uncommon that both airways are completely blocked simultaneously [94]. However, premature infants initiate compensatory respiration through the mouth before complete occlusion of the nose, and O_2 saturation is affected accordingly [95], suggesting that the negative impact on the oral function related to nasal obstruction may not require complete obstruction. Furthermore, under more natural physiological conditions, the incidence of nasal obstruction is probably more subtle and progressive, leading to more gradual adaptive responses [95]. Finally, the present neonatal rat model does not take into consideration the timing in which nasal obstruction occurs during early development. Postnatal development is indeed heterogeneous in relation to the various environmental challenges that neonatal organisms have to face, some periods and functions being potentially more sensitive than others. Nevertheless, if the above model of nasal occlusion has obvious limits to its generalisation, it reveals a complex pattern of interrelated effects involving all reactive abilities of neonatal and infantile organisms and raises important issues that can be generalised.

4. Discussion: Consequences for Human Neonates

What the above neonatal rat model teaches us, backed by extensive clinical observations in humans, is that the nose is more than a simple duct directing air to the lungs. From the very first breath (and perhaps before [13]), it also services sensory processes that are involved in the regulation of respiration (through trigeminal sensation) and of general behaviour mediated by the mouth (feeding motivation, orientation, and learning based on olfaction). At least in newborn and young mammals, the mouth has been emancipated from any involvement in respiration, leaving it reserved for ingestion, exploration, and communication. When incidental nasal obstruction occurs, all these functions are deferred in favour of maintaining air supply to the lungs. This change is far from benign as more than one-third of rat pups died from a 3-4-day nose obstruction in their second week of life. Such a high cost is fortunately not evident in humans. Neonatal and infantile organisms express considerable flexibility, as illustrated here by the outbreak of an abrupt shift to oral breathing in the neonatal rat model,

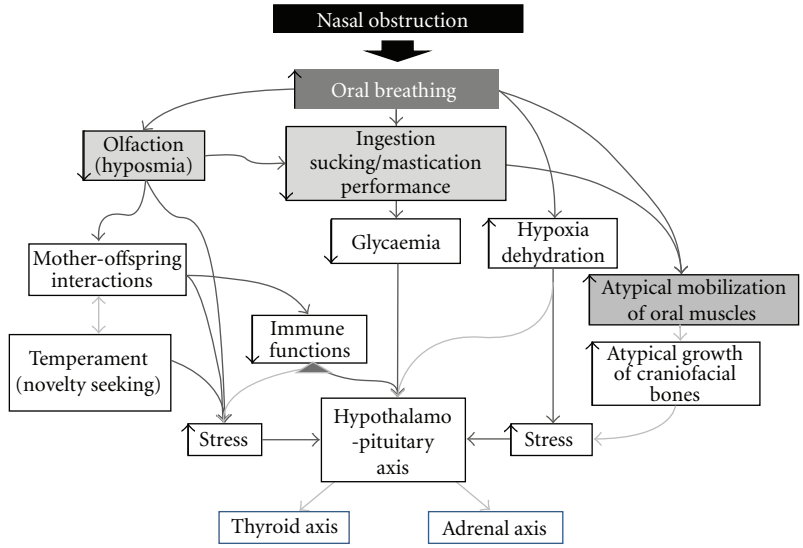

Figure 2: Representation of the main structures and processes involved in an episode of nasal obstruction in the neonate rat model.

there are functional limits and ceiling effects that need to be better understood in human infants.

A first major effect of this competition between respiration and ingestion at the mouth level is a reduced and disorganized sucking performance and a deprivation of sensory inputs to the developing olfactory tracts. It cannot be excluded that the dehydration incurred to oral and lingual mucosae by oral respiration may also affect gustatory abilities. Another major effect of the obligation to maintain breathing through the mouth is an altered oral competence in terms of muscular resistance and atypical shaping of the orofacial skeleton. Finally, early nasal obstruction or reduced patency has long-term consequences on biological rhythms and stress reactivity which, to our knowledge, have not yet been explored in human infants.

The other lesson derived from the neonatal rat model of nasal obstruction is that the organismal design is made of layers of adaptation, each with its own plasticity range and dynamics. Organisms can recruit various self-regulation processes to cope with challenges at different rates, and structures, forms, and organ compositions are induced by such challenges. Oral breathing (mouth opening) is the rapid response to nasal closure that also affects later the composition of the oral muscles mobilised by this new situation and the bones that support them. Then homeostasis of all endocrine systems is shifted towards maintaining energetic metabolism, hydration, growth, and stress response within limits. While some effects show rapid reversibility, others are slower to return to normal and others are nonreversible. Long-term consequences of nose blocking revealed by the rat pup model are related to the formation of the skull and oral structures, and to general reactivity. While the former long-term effects of nose obstruction have been described in human infants, the latter effect does not seem to have attracted much clinical attention. Finally, being exposed to the distress caused by a blocked nose may have variable consequences in relation to the subject's age and maturation. This is another point worthy of interest in human infants.

To summarise, organisms are integrated entities, and a function cannot be considered in isolation from the others. Thus, a change in oral function, even if it is only temporary, has repercussions on local and general functions. Such a change may be especially notable in more immature (namely, preterm) infants who must develop the skills needed to initiate oral feeding prior to progressing to coordinated sucking, breathing, and swallowing [96].

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Research Article

Prenatal Mouth Movements: Can We Identify Co-Ordinated Fetal Mouth and LIP Actions Necessary for Feeding?

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Observations of prenatal movement patterns of mouth and lips essential for feeding could have the potential for an assessment of the readiness to feed after birth. Although there is some research on sucking *per se*, we know very little about prenatal preparatory movements for sucking, namely, the ability to co-ordinate opening the mouth widely and then pursing the lips as if around a teat or nipple *in utero*. The purpose of the present study was to test two hypotheses using an adapted version of the Facial Action Coding Scheme: first that mouth stretch (AU 27) will be followed by lip pucker (AU 18), and second that these coordinated movement patterns will increase as a function of gestational age. Fifteen healthy fetuses were scanned four times between 24 and 36 weeks gestation using 4D ultrasound visualization. Results showed a decreased number of mouth stretches with increasing fetal age. Contrary to our expectations, we did not find an increase in movement patterns of mouth stretch followed by lip pucker in preparation for feeding *ex utero*. The results are discussed in terms of sensory triggers *in utero* required to elicit preparatory movements for feeding *ex utero*.

1. Introduction

In order to feed from the breast or an artificial teat, new-born infants need to be able to orally grasp and suck. Nutritive sucking has been examined in preterm infants [1] as a means of evaluating developmental risk for poor motor maturity [2]. The difficulty of many preterm infants to suck from the breast or a bottle has been attributed to their underdeveloped motor abilities due to their premature birth [3]. However, others suggest that it might be caused by neurological problems, such as not being able to co-ordinate breathing, sucking, and swallowing [4]. Medoff-Cooper et al. [5] examined the relationship between the pattern of sucking behavior in preterm infants and neurodevelopmental outcomes during the first year of life and found that sucking behavior in preterm infants is an indication of their psychomotor and mental development.

The majority of fetal movement patterns develop during the first half of pregnancy [6] and progress to mature forms after birth [7–9]. Observations of prenatal movement patterns of mouth and lips essential for later feeding ability could have the potential for an assessment of the readiness to suck after birth [10]. Research indicates that by 24 weeks of menstrual age, the fetus responds to palmar stimulation with mouth opening [11]. Humphrey [11] used direct observation of externalized human fetuses placed in a warm fluid bath showing a link between manual and oral activity. In sum, although some research [8–10] has addressed sucking behavior, such as, tongue movements and swallowing, less is known about prenatal preparatory movements for sucking [10], specifically the ability to coordinate mouth and lip movements *in utero*. In one study [10], fetal movements were observed in clusters so that, for example, mouthing was defined as "rhythmic open-and-close mouth movements without significant fluid or tongue movement" (page 68). In contrast to this work, the current study identified two specific movements, namely, the "pursing of lips" (AU 18) which does do not include inferior maxilla movement, whereas

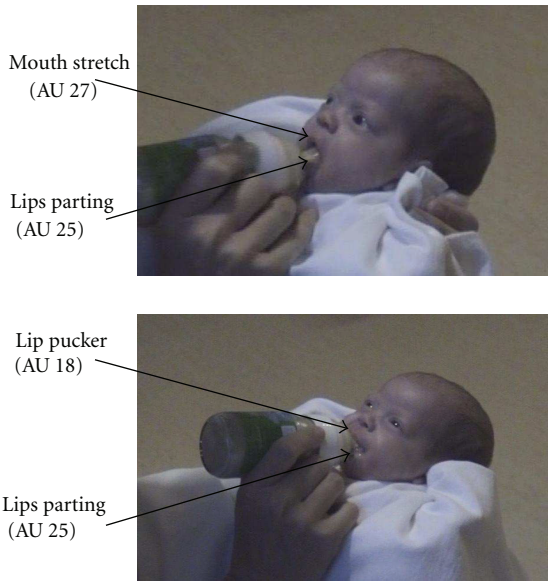

Figure 1: Mouth stretch and lip pucker during feeding in a premature infant aged 35.5 gestational weeks.

"mouth stretch" (AU 27) included a vertical stretching of the mouth by moving the inferior maxilla downward.

A number of studies have documented the development of oro-facial movements in the fetus (e.g., [10–15]), including mouth opening which has been observed in the fetus at 7 to 8 weeks of gestation, sucking at 15 weeks, and swallowing of amniotic fluid at 12 to 14 weeks. Yan et al. ([14]: page 112) defined sucking, observed in 6 out of 10 fetuses once or twice as "a series of movements of the jaws accompanied by the sinking of the cheeks toward the oral cavity, with the fingers always in the mouth." This contrasts with our observations of the unobstructed fetal face which did not include any stimulation of the oral region. Additionally, some researchers have examined jaw and tongue movements (e.g., [15]). Mizuno and Udea [4] assessed sucking performance in premature infants weekly between 24 to 36 weeks postconceptual age and suggested that the normative data collected on healthy premature infants could serve as a measure to identify preterm infants with sucking difficulties. They reported a significant correlation between premature infants' sucking behavior and performance at 18 months of age on the Bayley Scales of Infant Development which measures infant general psychomotor development. In contrast to investigations on sucking behavior, very little is known about labial and mouth movements essential for sucking and conducive to seizing the breast or artificial teat of a bottle.

1.1. Procedure for Collecting the Data. The purpose of the present study was to establish whether we can observe preliminary mouth and lip movements necessary for breast or bottle feeding in fetuses using 4D ultrasound scanning techniques. Movements required to take a breast or bottle include opening the mouth widely and then closing the mouth around the breast or bottle which results in a puckering of the lips (see Figure 1). Hence, if mouth and lip

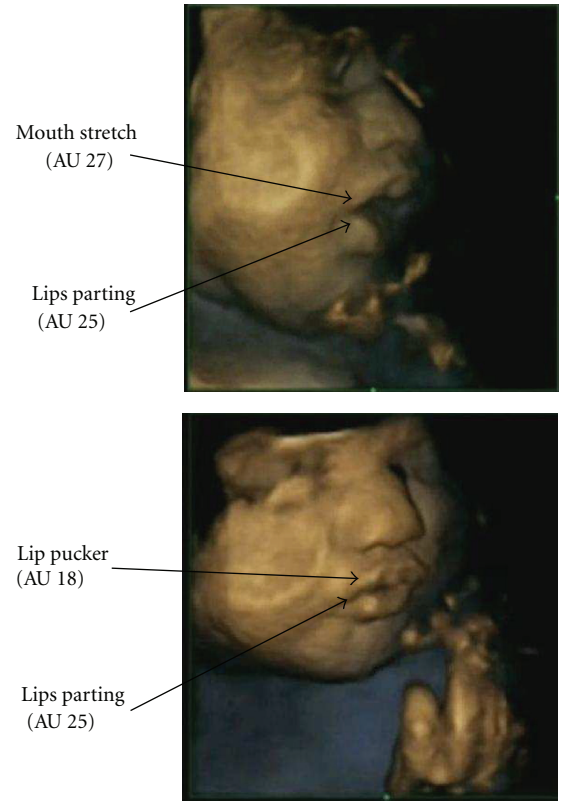

Figure 2: Expression of mouth stretch (AU 27) and lip pucker (AU 18) *in utero* in a fetus aged 33.1 weeks of gestation.

movements are preparatory for mature sucking abilities, we would expect firstly that mouth stretch (AU 27) would be followed by lip pucker (AU 18, see Figure 2) and secondly that these coordinated movement patterns would increase with maturation of the fetus. In order to test this hypothesis, fetuses were observed longitudinally from 24 to 36 weeks gestation in terms of two movements: opening the mouth widely into a mouth stretch and puckering the lips as if closing around a teat. If fetal mouth and lip movements are preparatory for sucking *ex utero*, we would expect as the fetus matures more of these movements to occur and to be coordinated in terms of mouth stretch (AU 27) being followed by lip pucker (AU 18).

2. Methods

2.1. Participants. Fifteen healthy fetuses, 8 girls and 7 boys, were scanned. The fetuses were observed four times at mean ages of 24.20 (range: 23.9–24.5 weeks), 28 (range: 27.8–28.2 weeks), 32.1 (range: 31.8–32.4 weeks), and 36.1 weeks (range: 36.0–36.4 weeks). All participants were first time mothers with a mean age of 27 years (range: 19–40 years), specifically recruited through the radiographers of the antenatal unit of the James Cook University Hospital, Middlesbrough, UK, following ethical procedures. At birth, the mean weight of the infants was 3283 grams (range: 2380–4160 grams). Mean Apgar scores measured at 1 and 5 minutes after birth were

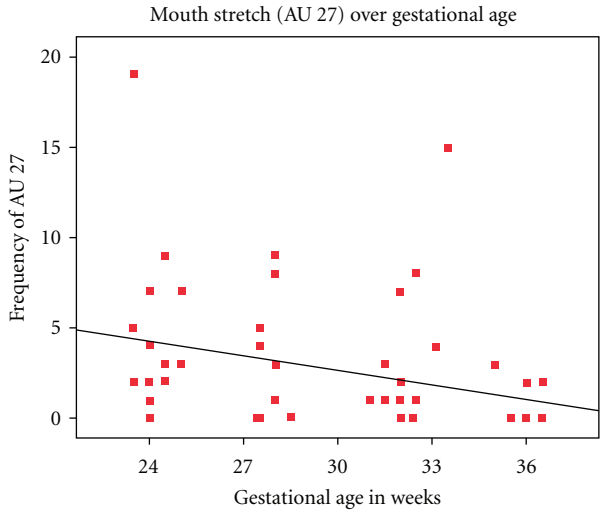

Figure 3: Correlation between the number of mouth stretch (AU 27) and gestational age between weeks 24 and 36. A decrease in mouth stretch frequency can be seen as a function of age.

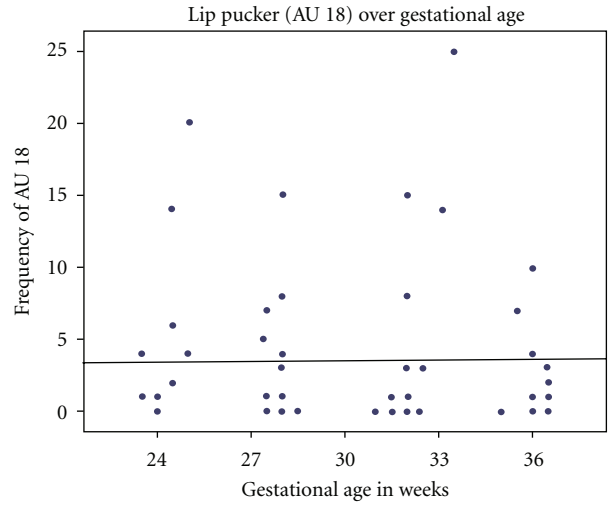

Figure 4: Correlation between the number of lip pucker (AU 18) and gestational age between weeks 24 and 36. Lip puckering frequency is stable as a function of age.

9.06 (range: 9–10) and 9.33 (range: 9–10). Ethical permission was granted by the County Durham and Tees Valley 2 Research Ethics Committee (REC Ref: 08/H0908/31), James Cook University Hospital. All mothers gave informed written consent.

After their 20-week anomaly scan had been completed, mothers were approached by a radiographer to seek consent to participate in the study. All participating mothers received four additional scans in the mornings after breakfast, similar in procedure to diagnostic scans they have to undergo at 12 and 20 weeks. During these scans, mothers lay supine either on their back or on the side, depending on the position of the fetus and the comfort of the mother. Fetuses were observed in 4D imaging while active (state 2F or 3F; [16]) for approximately 15 minutes. During consent and before each procedure mothers were made aware that these additional scans were performed for research purposes only and were not routine medical scans. All mothers were given a DVD copy of their scans.

The fetal face and upper torso were visualized by means of 4D full frontal or facial profile ultrasound recordings, and recorded for off line analysis with a GE Voluson 730 Expert Ultrasound System using a GE RAB4–8L Macro 4D Convex Array Transducer. For each observation period, we coded 10 minutes of scan (which were not necessarily consecutive) when the full face was visible, starting with the first moment when the face was codable.

The Facial Action Coding System (FACS, [17]) developed for adults is an anatomically based system, itemizing facial muscle movements, or "action units" (AUs). This system was adapted for fetal facial movements [18] using a method designed for the eye brow region of the face [19] which defined movements in the upper face in relation to FACS [17], together with the web resource Artnatomy [20]. Using this new method, we identified two fetal mouth movements required for sucking from breast or bottle, namely mouth stretch (AU 27) "indicating the jaw is pulled down and the mouth is stretched open" ([21]: page 92) and lip pucker (AU 18) "drawing the lips medially, pursing or puckering them, causing the lips to protrude" ([21] page 233). Using Cohen's Kappa, reliability was established for these scans, which were coded independently by a new coder trained in this coding system. This resulted in reliability estimates for AU 27 and AU 18 (mean = .87, overall mean range: .81–.92).

3. Results

All fetuses showed mouth movements during the 10-minute scans. Specifically, for AU 27 (mouth stretch), the fetuses showed a mean frequency in the first scan of 4.40 (range: 0–19), in the second scan of 2.73 (range: 0–9), in the third scan of 3.07 (range: 0–15) and in the fourth scan of .990 (range: 0–3). A Kruskall-Wallis test ($X^2 = 16.374$; df = 3, $P < .001$) showed that in the four scans there was a significant difference in mouth stretch frequencies, with mouth stretch being most frequent at 24 weeks and least frequent at 36 weeks gestation.

In contrast, the frequency of lip pucker (AU 18) did not differ significantly over gestational age. For this AU, the fetuses showed a mean frequency in the first scan of 3.60 (range: 0–20), in the second scan of 3.20 (range: 0–15), in the third scan of 4.87 (range: 0–25), and in the forth scan of 2.47 (range: 0–10). A Kruskall-Wallis test indicated that over the 4 scans there were no significant differences in the frequency of lip pucker displayed ($X^2 = 0.41$; df = 3, $P = .998$, ns).

Finally, a correlation analysis indicated that the rate of occurrence of mouth stretch (AU 27) was negatively correlated with gestational age. As the fetuses developed from 24 to 36 weeks of gestation they displayed fewer mouth stretches (Spearman's $\rho = -.463$, $P < 0.000$, Figure 3). In contrast, lip pucker (AU 18) was not significantly associated with age (see Figure 4). Although, mouthstretch (AU 27)

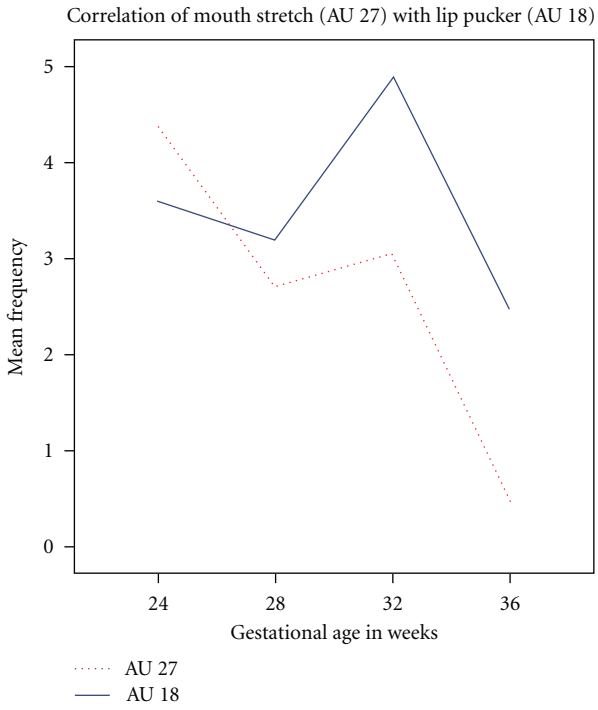

Figure 5: Frequency of mouth stretch (AU 27) and lip pucker (AU 18) observed between 24 and 36 weeks gestation.

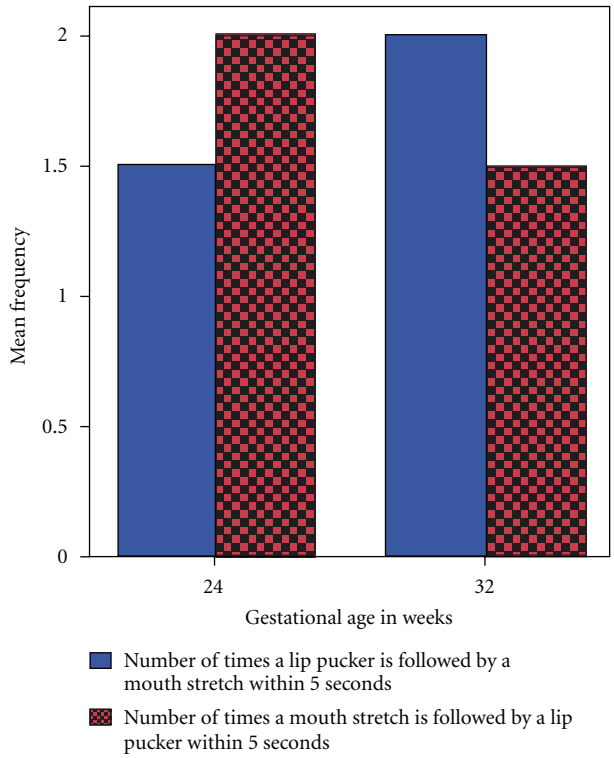

Figure 6: Mean number of mouth stretches (AU 27) followed by a lip pucker (AU 18), or conversely, within 5 seconds of observation in relation to gestational age.

and lippucker (AU 18) were correlated (Spearman's $\rho = .322$, $P < .005$; see Figure 5), fetuses showed very few instances of mouth stretch followed by lip pucker (see Figure 6) and these were unrelated to fetal age.

4. Discussion

Research on general fetal movements indicates that with increasing gestation fetuses move less [16]. For example, Kurjak et al. ([22]: page 25), examining facial expressions such as grimacing, mouthing, and yawning, noted "a tendency towards a decreased frequency of observed facial expressions with increasing gestational age." This is also reflected in the present findings on mouth stretch. Our sample of fetuses observed from 24 to 36 gestational weeks showed decreasing numbers of mouth stretches with increasing fetal age. In contrast, the frequency of lip pucker movements, occurring rather less frequently than mouth stretches, was relatively stable over time.

Contrary to our hypothesis of an increase in movement patterns of mouth-stretch followed by lip pucker in preparation for feeding *ex utero*, we found only very few instances of the sequence of movements, and these were not related to fetal gestational age. Hence, it seems that the feeding preparatory movement pattern of mouth stretch (AU 27) followed by puckering the lips (AU 18) does not occur more frequently as the fetus grows older. This could be because we observed fetuses moving their mouth without an object stimulating their lips. Given that mothers were observed in the morning after breakfast, although we did not ascertain what they ate before coming to the clinic, the prandial state of the mother is unlikely to have influenced fetal mouth movements differentially. Although Yan et al. [14] observed fetuses while having a finger in the mouth, which could be seen once or twice in 6 out of 10 fetuses, they did not observe a sequence of movements. In order to examine a situation in which the fetus touches his or her lips *in utero* and thereby provides a stimulus akin to a bottle or breast, we currently investigate whether they perform more of the pattern of movements required for oral grasping movements after tactile stimulation.

Fetal movement patterns which can be seen in the first half of pregnancy [6] develop to mature forms after birth in relation to appropriate stimulation [7]. For example, although certain movements, such as knee jerks, can be observed to occur spontaneously prenatally, they cannot be elicited *in utero* [23]. After birth, however, the infant's vestibular responses to stimulation, such as the Moro response, can be clearly elicited [24]. The sensory trigger mechanism of movement patterns which can be observed to occur spontaneously *in utero* becomes mandatory in the postnatal adaptation of the newborn infant [25]. For example, although the fetus ingests amniotic fluid whenever sucking movements occur [10, 24], after birth sucking behavior needs to be triggered by specific stimuli afforded by the actual feeding situation. Hence, it is a matter of vital biological adaptation that sucking is elicited by touching the lips of the newborn baby in order to initiate feeding [24]

and could be the reason why we did not find the expected movement pattern. In contrast, in premature infants, as illustrated in Figure 2, we can observe functional puckering of lips when the teat of the bottle is held in place.

In sum, the present study demonstrates that the movement pattern of mouth stretch (AU 27) followed by lip pucker (AU 18) can be observed prenatally. However, in the absence of appropriate stimulation, we did not observe an increased frequency of these sequential movement patterns with advancing gestation. Future research needs to address the question of whether such a movement pattern can be observed prenatally with stimulation of the oral/nasal region by either touch or chemoreception.

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Clinical Study

A New Bottle Design Decreases Hypoxemic Episodes during Feeding in Preterm Infants

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Oxygen saturation is lower during bottle feeding than during breastfeeding in preterm infants. Our objective was to compare two different bottle systems in healthy preterm infants before discharge in terms of SpO_2 and oral feeding efficiency (rate of milk intake). Infants without supplement oxygen needs were evaluated twice on the same day during two consecutive feeds, by the same nurse. Infants served as their own controls for comparison of two systems of bottles, the order of which was randomized. The new bottle's nipple design mimics mom's breast in shape and feel, and the bottle vents to air when the child sucks on the nipple. The other system was the hospital's standard plastic bottle with silicone nipple. The rate of milk intake was calculated as the total volume transferred minus volume lost divided by time of feeding, mL/min. Thirty-four infants (BW: $1,163 \pm 479.1$ g) were studied at 35.4 ± 1.3 weeks after-conception. SpO_2 was significantly higher in infants fed with the new bottle design. Milk intake rate was significantly higher with the new bottle than with the standard bottle design. The new bottle design improves oral feeding performance in preterm infants near to discharge when compared to that of a standard bottle.

1. Introduction

Preterm infants who receive expressed breast milk rather than formula have fewer infections and necrotizing enterocolitis, as well as other better developments [1]. However, mothers of vulnerable infants, such as neonates, encounter a variety of unique breastfeeding barriers and challenges that may result in a decreased rate of breastfeeding. In these cases, the next "best" alternative is bottle feeding with expressed breast milk. However, the characteristics of commercial bottle systems vary widely in terms of flow rates achieved, and some may require well-developed sucking capabilities—which may be problematic in premature babies. The main purpose of this research is to provide much needed information on how a premature baby tackles being fed expressed milk from different bottle systems in terms of oxygen saturation (SpO_2) and oral feeding efficiency.

Improving oral feeding skills accelerates attainment of independent oral feeding, shortens hospitalization, and reduces medical costs, allowing earlier family reunification and development of more appropriate mother-infant interaction and bonding. Infant feeding is a complex process, requiring the precise coordination of sucking, and pharyngeal swallowing and breathing which are mutually exclusive. Therefore, the pharynx must be continually and rapidly reconfigured so that baby can successfully eat and breathe almost simultaneously. Unlike artificial nipples, the human breast transforms to fit the shape, size, and positioning of the infant's mouth. In addition, the magnitude and consistency of milk flow from a milk bottle are quite different from that of the breast, and milk bottles may generate excessive internal negative pressure, which may collapse the nipple and provide resistance to infant sucking.

Because oxygen saturation is higher during breastfeeding than bottle feeding [2, 3] there may be a mechanistic basis for the advantage from differences in tongue posture and less disruption of breathing. By extension, there may be

differences among bottle feeding systems, some of which may promote more natural postures and breathing patterns than others. Breastfeeding involves tongue and jaw movement to "strip milk" from the breast's natural milk ducts, while bottle-feeding involves sucking. Breastfed babies do not learn to release, gasp, and reattach while feeding because the breast does not build up an internal vacuum, nor does it collapse. This is one of the reasons why breastfed babies have such a hard time transitioning to a regular baby bottle. When babies nurse from regular reusable bottles, vacuum builds up within until the nipple collapses on itself. Babies who are bottle-fed learn to release the nipple and gasp air through their nose and mouth and then resume sucking on the bottle.

Our hypothesis is that use of a new bottle system (developed by MAM Babyantikel GesmhH) when compared to our hospital's standard firm-silicone bottle (without valve and vents and with traditional bottle nipple shape feeling unlike mother's breast) will reduce stress of oxygen desaturation and improve feeding outcome. We compare and evaluate them to judge which comes closest to "gold-standard" breast feeding. The new MAM bottle features the ULTIVENT valve and nipple, which interact with each other to replicate breastfeeding.

2. Methods

2.1. Subjects. Infants were recruited from the Neonatal Intensive Care Unit (NICU) at Hospital Italiano of San Justo Agustin Rocca, Buenos Aires, Argentina. A total of 34 clinically stable preterm neonates, postconceptional age (PCA) >34 weeks, were chosen as eligible for the study. They were near the time of discharge from the NICU, were not receiving oxygen, and were exclusively oral-feeding 100% of their daily milk intake. These preterm infants were evaluated and enrolled after obtaining written informed parental consent if they were (1) born with >27 weeks gestational age (GA) as determined by obstetrical ultrasound and clinical exam; (2) without medical problems that might influence feeding; (3) of appropriate size for GA; (4) without known congenital anomalies; (5) without chronic medical conditions including bronchopulmonary dysplasia, intraventricular hemorrhage grade III or IV, periventricular leukomalacia, or necrotizing enterocolitis; (6) not exclusively breastfeed; (7) free of any observed episodes of apnea, bradycardia, or significant oxygen desaturation prior to evaluation.

2.2. Physical Properties of Baby Bottles

2.2.1. Standard Bottle (Figure 1). The standard bottle used in this study was straight and cylindrical in shape, without valve and vents and with traditional bottle nipple shape feeling unlike mother's breast. Vacuum builds up within the bottle as an infant withdraws milk. As feed progresses, this negative pressure/force increases, opposing the suction exerted by the infant. This situation leads to difficulty in generating suction and/or results in a decrease in the rate of milk flow.

Figure 1: Standard bottle.

2.2.2. New Bottle Design (Figure 2(a)) and New Nipple Design (Figure 2(b)). The shape, design, placement, and construction of the valve within the new bottle design allow for a continuous and ultrasensitive response, preventing a buildup of internal vacuum. The center of the valve opens and closes to release vacuum from the bottle in a continuous process during nursing. This continuous opening and closing process is what allows the nipple to react like the breast. It allows the nipple to deliver milk in an uninterrupted process just like the breast during breastfeeding.

For the same sucking effort the new bottle will give a higher flow rate because the atmospheric pressure in the bottle above the milk will help push the milk whereas a partial vacuum above the milk will retard the flow to baby.

The newly designed nipple (Figure 2(b)) mimics mom's breast in shape and feel. The breast-shaped nipple contains internal Rib structures to encourage "stripping" action and eliminate nipple collapse. The silky textured area replicates the silky soft skin of the mother's breast. The broad bulge of the teat allows sufficient space for the lips to latch on, just like the breast, so the baby can uninterruptedly feed without swallowing air.

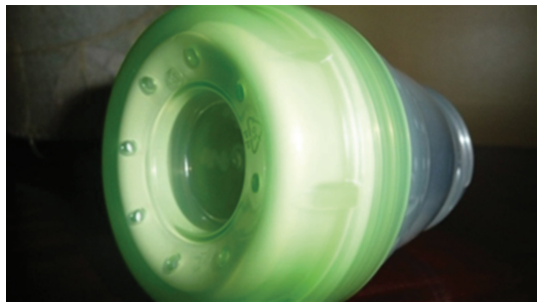
The new bottle was tested by using a sucking device (Figure 3).

The main features of the apparatus are as follows.

The sucking force generated by the *Vacuum Pump* may be accurately varied to within 1 mmHg. It is connected to the *Receiver* and hence allows suction to be applied to the *Adaptor* and *Feeding Teat*.

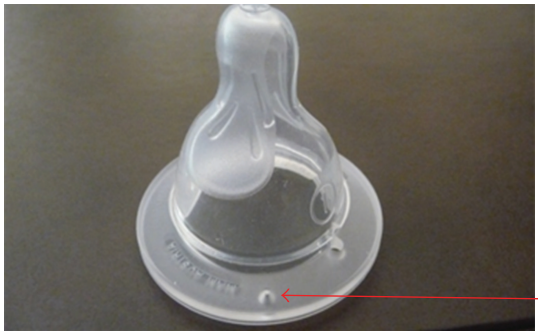
The *Feeding Teat* is held in place in the Receiver with an *Adaptor* designed for the shape of the particular feeding teat being tested.

Via a hole drilled in the bottom of the standard bottle or side of the new *Bottle*, the internal negative pressure (or vacuum) in the bottle can be continuously



The bottom of the new bottle contains the sensitive valve which opens up when the baby sucks, so there is virtually no vacuum buildup.

(a)



The teat is also vented with a little hole and/or valve in the flange (rim) of the teat.
This adds to the venting of the bottle.

(b)

Figure 2: (a) New Bottle design. (b) The new bottle's nipple.

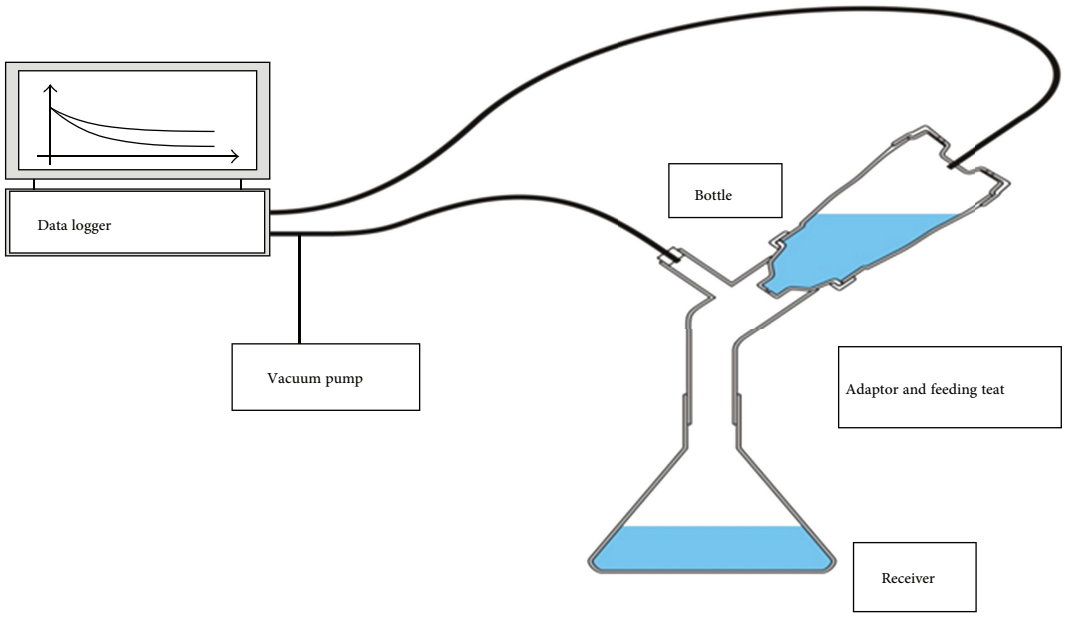


Figure 3: Schematic view of the bottle testing apparatus.

recorded in a *Data Logger* which is also designed to record the sucking pressure exerted by the vacuum pump.

Tap water was used as a simulant for milk throughout the tests.

The red trace in the charts below is the "Sucking pressure" exerted by the vacuum pump on the bottle.

The blue trace (called by the data logger "Measuring pressure") is a measurement of the internal negative pressure in the air space in the bottle.

Two representative tests are presented as examples.

This uses a sucking pressure of up to 150 mm. The internal pressure of the bottle quickly falls (~110 mmHg after 10 seconds) and reaches a minimum after 27 seconds (see Figure 4).

The fall of internal pressure is much slower (circa 25 mmHg after 10 seconds).

The bottle ventilation initiates after 18 seconds at −38 mmHg and maintains a slightly reducing negative internal pressure (−33 ± 1 mmHg) for the remainder of the trial (see Figure 5). What Is the Difference with Feeding Function between the New Bottle Design and the Standard Bottle in terms of the Function of Mother's Breast?

The physiological pressure in the milk duct forcing out the milk from the nipple remains the same high value while baby is sucking. This is similar to the new bottle's valves which keep atmospheric pressure above the milk the same. So the new nipple correctly mimics the mom's constant breast pressure difference.

3. Study Design

Infants were evaluated twice on the same day during two consecutive feedings by the same research nurse, who was blinded to the oxygen saturation (SpO_2) recording. They were studied five minutes before and throughout a bottle feeding at 3 h intervals using the two different bottles: a new design bottle/nipple or a conventional bottle/nipple. The sequence in which these bottles were given was determined using a random allocation procedure. Infants served as their own controls for comparison of bottle feedings and all feedings were performed with their mother's expressed milk. For cases in which the amount of mother's milk was insufficient, the volume was supplemented with a milk formula adequate for preterm infants. To ensure that we did not interfere with breastfeeding, assessments were made when mothers were absent.

4. Oral Feeding Outcomes

The principal aim of our study was to compare the effects of bottle-feeding using a new bottle design (MAM Ultivent bottle) versus the hospital's standard bottle on the occurrence, severity, and pattern of oxygen desaturation in healthy preterm babies near the time of discharge from the NICU. The secondary outcome was a comparison of the oral feeding efficiency during the use of the two systems of bottle feeding. Oral feeding efficiency was measured by the rate of milk intake and percent milk leakage or loss.

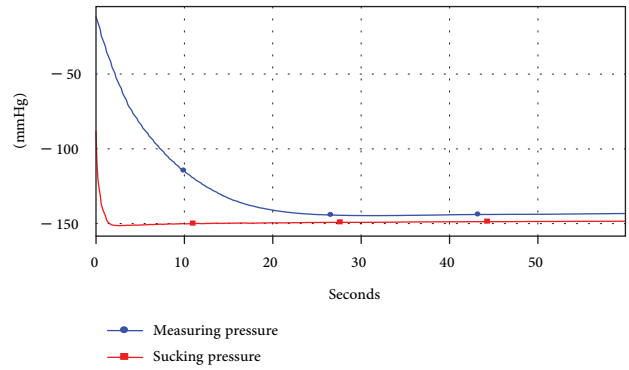


Figure 4: Performance of conventional unventilated bottle with an unvented teat.

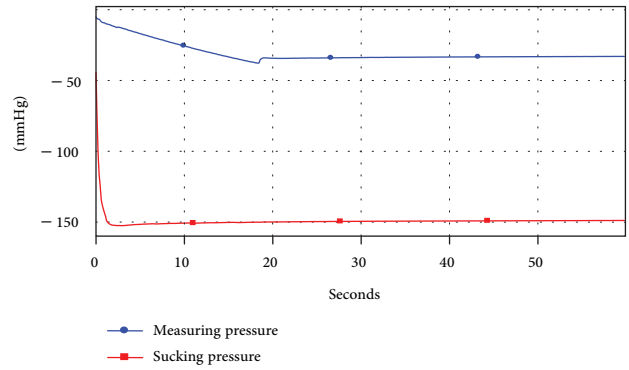


Figure 5: Performance of new bottle design with a vented teat.

5. Data Collection

SpO_2 was recorded continuously with the Nellcor pulse oximetry channel of the EdenTrace II Plus AMS (Mallinckrodt, St. Louis, MO). Data were stored and recovered for analysis. The oximeter probe was placed on the infants' foot in a position that provided an optimal signal. Clinically significant desaturation events were defined as any decrease in SpO_2 below 90% for 1 s or more. In addition, instantaneous severe drops in SpO_2 were considered artifacts. We considered the following desaturation variables: (1) the percentage of feeding time SpO_2 < 90%; (2) percentage of feeding time SpO_2 90%–94%; (3) the number of desaturation events per infant feeding; (4) the time with SpO_2 < 90% and (5) mean SpO_2 during feeding. The average rate of milk intake (mL/min) was considered as the total volume transferred minus volume lost during feeding divided by the feeding time which was defined as the time from the first nipple in mouth through the final nipple out. The duration of feeding included the "out" times during which the infant needed

to burp, cough, or rest. These decisions were left to the discretion of the research nurse who assessed infant's need. The percent milk leakage or loss was measured by weighing a bib before and after each feeding (an increase of 1 g = 1 mL of loss) over the total volume of milk removed from the bottle.

6. Sample Size

Taking into account that NICU infants currently spend 20% of their bottle feeding time with SpO_2 levels <90% [4], our expectation is that infants fed with the new MAM Ultivent system will increase the oxygen saturation sufficiently so the infants will spend 10% or less of their time with SpO_2 levels <90%. A sufficient sample size of 34 preterm infants was calculated with an alpha level of 0.05, using a type 1 error of 0.05 and a power of 0.80.

7. Statistical Analysis

Data were analyzed with the Epi-Info 6.02, Stata 5.0. Descriptive statistics were calculated for infant and maternal characteristics (baseline SpO_2,body weight (BW), gestational age (GA), PostConceptionalAge (PCA)) and maternal parity, feeding length, and desaturation variables. The Student's *t*-test was used to analyze data showing a normal distribution and the nonparametric Wilcoxon test was used for variables that did not show a normal distribution. In this last case results are presented as medians and interquartile ranges.

8. Ethical Considerations

The study was approved by the Hospital Institutional Review Board for Protection of Human Subjects, and a signed written informed parental consent was obtained before each study pair of feedings.

9. Results

Thirty-four preterm infants were studied. Infants and maternal characteristics are summarized in Table 1. Infants did not differ in PCA at the time of the study (range 35-36 weeks) and were near discharge (mean 2.4 days). Baseline SpO_2 was within a clinically acceptable range.

9.1. Desaturations Events. Descriptive data of the desaturation events are provided in Table 2. Preterm infants who are normally oxygenated in room air have significant desaturation during bottle feeding. However, taking into account all the desaturation variables, the SpO_2 during feeding was significantly higher in infants fed with the new bottle design compared with the standard bottle. Of all the minutes of the feeding, the initial minute of the feeding had the highest number of desaturations both with the new design bottle as with the standard bottle. There was no significant effect of feeding bottle on the frequency of apnea or bradycardia.

Table 1: Characteristics of the sample ($N = 34$): 14 males, 20 females.

Variables	Mean	S.D.
Infants		
BW (g)	1163.4	479.1
GA (weeks)	31.1	3.1
PCA (weeks)	35.4	1.3
Days on oxygen (prior to study)	6.3	10.4
Days before discharge	2.4	1.61
SpO_2 during baseline period (%)	96.8	1.90
Mothers		
Age (years)	29.06	4.72
Education (years)	13.93	3.41
Parity	2	1.8

9.2. Oral Feeding Outcomes. Rate of milk transfer was statistically different between the two bottle nipples. Rate of milk transfer (Figure 6) was significantly less with the standard bottle than with the new bottle design (5.0 ± 2.2 Versus 6.9 ± 2.6 ml/min, resp., $P < 0.0001$).

Percent milk loss (Figure 7) decreased with the new bottle design when we compared it to the standard bottle (3.5 ± 4.3 Versus. $5.4 \pm 5.6\%$, resp., $P < 0.0001$).

10. Discussion

Preterm infants who are normally oxygenated in room air may have significant desaturation during bottle feeding [5]. With the increased survival of preterm infants, awareness of oral feeding difficulties in this population is growing. A temporal association between feeding and episodes of cyanosis in preterm infants was first noted more than 90 years ago [6]. In the present study, we confirm our hypothesis that the use of the new design bottle when compared to that of standard bottle improves oral feeding performance in infants born with 27 week gestation or more in terms of oxygen saturation and oral feeding efficiency, that is, greater rate of milk intake and less percent milk leakage/loss.

Oxygen desaturation during bottle feeding is impacted by the feeding condition and the health of the infant. Previous studies have consistently demonstrated that breastfed babies have higher oxygen saturation than bottlefed babies. A frequently cited explanation for this difference is that bottle-feeding may promote a higher rate of swallowing and, in turn, more frequent interruptions of breathing. Indeed, studies have shown that there is less ventilatory disruption during breast-feeding compared with bottle feeding, which may result in higher oxygen saturation [7].

Infants with lower oxygen saturation tend to have shorter sucking bursts, potentially signifying less energy available for sucking. They also tend to organize restorative breathing between sucking bursts poorly, engaging instead, in shorter intervals between sucking bursts [8].

We have demonstrated that VLBW infants continue to have frequent oxygen desaturation events during bottle

Table 2: Oxygen desaturation events, expressed as median and (interquartile range).

	Standard bottle	New bottle design	P^*
Percentage of feeding time SpO_2 <90%	8% (3–13)	5% (2–11)	<0.004
Percentage of feeding time SpO_2 90%−94%	13% (6–21)	8% (2–18)	<0.0007
Number of desaturation events per infant	10 (1–19)	4 (1–8)	<0.001
Time with SpO_2 <90% (s)	46 s (8.3–150)	30 s (6–96)	<0.001
Mean SpO_2 during feeding	94 (91–96)	96 (93–98)	<0.0008

*Wilcoxon signed-0 rank test.

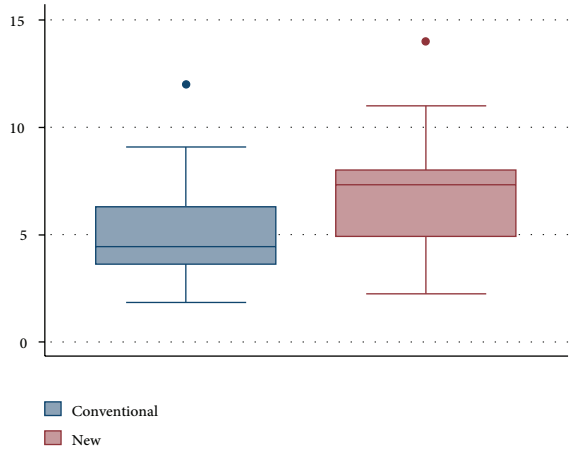


Figure 6: Rate of milk transfer (mL/min).

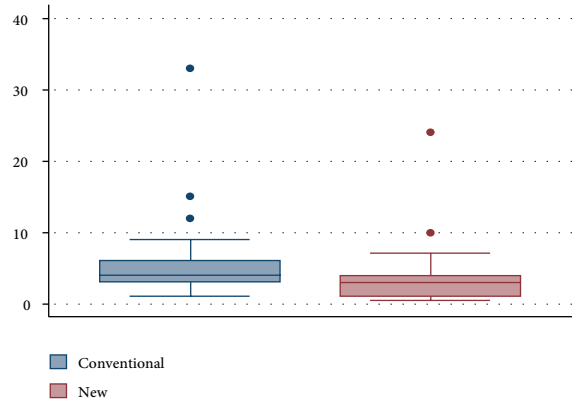


Figure 7: Percent milk loss.

feeding near the time of discharge from the NICU. Thoyre and Carlon have demonstrated in an elegant paper that preterm infants at the postconceptional age of 36.5 ± 1.6 weeks spent on average 20% of their bottle feeding time with oxygen levels below 90%, near the time of discharge [4]. These values are significantly lower than the normal range of oxygen saturation for preterm infants during non-feeding periods. The preterm infants included in our study showed higher oxygen saturation during the feeding period compared with Thoyre and Carlson's study [4]. This fact is explained because they included 30% of their patients with supplemental oxygen requirements. Our study population has included only healthy preterm infants prior to discharge from the hospital. We speculate that preterm infants showed less desaturation events during the feeding process with the new design bottle because the nipple resembles the mother breast in terms of shape and texture of the silicone, that is, much softer and silkier in comparison to the standard nipple. The shape, design, placement, and construction of the valve within the new design bottle (a large vent with a large center hole) prevent a high buildup of internal vacuum. Adequate oxygenation enables infants to maintain behavioral organization [9]. Lower oxygen saturation during feeding impacts the infant's ability to organize and maintain oral feeding skills. The low oxygen saturation observed in the infants when they were fed with the standard bottle explains the poorer feeding outcomes in regards to the preterm infants fed with the new design bottle.

In summary, although breastfeeding is clearly best for infants, it may not always be possible. Our results suggest that the overall feeding pattern and oxygenation of the new model design bottle are closer to the physiologic norm than the standard bottle. The new design bottle would be very useful for some preterm infants, particularly those with bronchopulmonary dysplasia that exhibit significant desaturation during and immediately after bottle feeding [10]. The results of this study have several practical implications for evaluating issues of great concern to women who breast-feed but who may choose to intersperse some bottle and artificial nipple feeding with breast milk while they are at work.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgment

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Review Article

Oral Feeding Competences of Healthy Preterm Infants: A Review

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Background. With increasing sophistication and technology, survival rates hugely improved among preterm infants admitted to the neonatal intensive care unit. Nutrition and feeding remain a challenge and preterm infants are at high risk of encountering oral feeding difficulties. *Objective.* To determine what facts may impact on oral feeding readiness and competence and which kind of interventions should enhance oral feeding performance in preterm infants. *Search Strategy.* MEDILINE database was explored and articles relevant to this topic were collected starting from 2009 up to 2011. *Main Results.* Increasingly robust alertness prior to and during feeding does positively impact the infant's feeding Skills. The review found that oral and non-oral sensorimotor interventions, provided singly or in combination, shortened the transition time to independent oral feeding in preterm infants and that preterm infants who received a combined oral and sensorimotor intervention demonstrated more advanced nutritive sucking, suck-swallow and swallow-respiration coordination than those who received an oral or sensorimotor intervention singly.

The mortality among preterm infants has dramatically decreased in the last decade in the developed countries. Very low-birth-weight (VLBW) infants increased their survival from 50% [1] to more than 85% [2]. However, these VLBW infants are at the greatest risk for medical and developmental sequelae related to their prematurity, which is frequently exacerbated by their prolonged staying in the newborn intensive care unit (NICU) [3–5]. These atypical early experiences alter their development and modify their behaviour [6]. Many clinical factors such as the development of chronic lung disease, recurrent apnoeas and bradycardia, nutritional deficits, as well as stressful environmental conditions, including constant noise, activity, and bright light, may act in combination to impact on the developing preterm infant brain [7]. NICU environment could influence the transition to independent oral feeding and thus delay hospital discharge, negatively affect mother-infant interaction and potentially lead to childhood feeding disorders. As a consequence, there is a need for efficacious early interventions to enhance preterm infants' oral feeding performance [8–10].

The relationship between the maturation of behavioural states, mainly alertness, and the acquisition of oral feeding would deserve investigation [11–16]. The development of this highly complex process reveals a great deal about the maturation of the developing brain and relationships between physiologic and behavioural indices. Among the huge number of routine caregiving interventions in the NICU, it is the successful initiation of oral nipple feeding and attainment of nutritive sucking competence that appears to be the primary determinant of discharge readiness. Attainment of independent oral feeding is one of the criteria recommended by the American Academy of Pediatrics for hospital discharge of preterm infants [17]. The infant's inability to wean from the tube feeding likely will delay hospital discharge and mother-infant reunion and will increase the hospital cost and maternal stress [18, 19]. The sophisticated integration of sucking, swallowing, and breathing with behavioural state is considered the "most highly organized behaviour of the young infant" [20]. There are two dilemmas caregivers face when addressing oral feeding difficulties, that is, infants' ability to complete their

feedings safely and the appropriate rate of advancement to independent oral feeding.

McGrath and Medoff-Cooper [21] examined the relationship between the maturation of alertness and the acquisition of nutritive sucking competence during the transition to oral nipple feeding in the NICU. Alertness and nutritive sucking are defined within the context of the synactive theory of development [22, 23]. The synactive theory of development is a model of neurobehavioral development in which the dynamic maturational process of behavioural organization in the preterm infant is illustrated. The integration of the physiologic and behavioural systems is presented within a framework that describes how the maturing infant balances input from the environment while coping with internal physiologic demands. Als et al. [24] have recommended the use of a developmental care approach to promote transition to oral feeding with the reasoning that if an infant's stability, organization, and competence could be enhanced, his/her physiologic and behavioural expression would be optimized. Als proposed that the extent of behavioural organization shown through the infant's unique pattern of behavioural cues and underlying physiologic stability is an indication of central nervous system maturation. Infant behavioural state is starting to be recognized as an important variable in routine caregiving interventions [25]. Alertness is a behavioural state in the normal newborn that is critical to interaction with the environment and has been linked to later cognitive learning and development [26–29]. Typically, caregivers determine oral feeding readiness based on physiologic indicators such as successful weight gain and respiratory stability [30–32]. Postmenstrual age (PMA) or behavioural maturation is often only considered after these other indicators have been satisfied. The infant's ability to reach and maintain robust alertness is seldom used as a meaningful assessment parameter in relationship to oral feeding readiness in the NICU. This study shows that increasingly robust alertness prior to and during feeding does positively impact the infants' feeding competence especially in their ability to generate numbers of sucks and numbers of sucks per burst. Furthermore, the results provide strong support for using the ability to reach and maintain robust alertness as a meaningful readiness parameter for oral nipple feeding.

Oral feeding requires coordination of nutritive sucking, swallowing, and breathing as well.

Oral feeding is not initiated in preterm infants before 32 weeks of PMA mainly because the coordination of sucking, swallowing, and respiration is not established. According to some authors, rhythmic breathing during feeding is first acquired between 34 and 36 weeks' PMA, simultaneously with the maturation of other physiologic processes [33]. In their study, Mizuno and Ueda aimed at establishing normative maturational data for feeding behaviour of preterm infants from 32 to 36 weeks of PMA and evaluating how the relation between swallowing and respiration changed with maturation.

They found that feeding behaviour in preterm infants matured significantly between 33 and 36 of weeks PMA, and swallowing infrequently interrupted respiration during feeding after 35 of weeks PMA. Before 34 weeks' PMA, swallowing occurred usually during a respiratory pause, and after 35 weeks' PMA, swallowing occurred typically at the end of inspiration. They also demonstrated that there was a significant difference in the coordination of swallowing and breathing between <34 and >34 weeks' PMA. The respiratory rate reduction during intermittent sucking after 34 weeks was smaller than that at 32 or 33 weeks' PMA. In preterm infants the maturation in respiration during feeding is not established fully yet at 36 weeks' PMA [34].

Sucking is one of many factors involved in oral feeding [35], and suck-swallow-respiration coordination is a critical factor in achieving safe and successful oral feeding in preterm infants. Sensorimotor intervention is used to improve oral feeding, that is, the provision of developmentally appropriate oral, tactile, kinaesthetic, vestibular, and auditory inputs to facilitate the development of existing skills [36–39]. Oral feeding is a complex multisystem process that involves both the oral and other systems, including cardiorespiratory, gastrointestinal, and neurological [40]. In their study, Fucile et al. aimed at determining whether oral, tactile/kinaesthetic, or combined (oral + tactile/kinaesthetic) interventions enhance oral feeding performance and whether combined interventions have an additive and/or synergistic effect [41]. The authors came to the conclusions that oral and nonoral sensorimotor interventions (i.e. oral and tactile/kinaesthetic) accelerated the transition from introduction of oral feeding to independent oral feeding and enhanced oral feeding skills; sensorimotor interventions had beneficial effects beyond the specific targeted system; combined (oral + tactile/kinaesthetic) sensorimotor intervention had an additive and/or synergistic effect on oral feeding performance over single sensorimotor interventions. Specifically, all three interventions improved proficiency (percent volume taken in first 5 minutes), volume transfer (percent total volume taken), and rate of transfer (mL/min), compared to controls. In conclusion, oral and nonoral sensorimotor interventions provided singly or in combination shortened the transition time to independent oral feeding in preterm infants. These findings demonstrated that sensorimotor interventions have beneficial effects beyond their specific site of input. One limitation of this study was that the three interventions did not reduce the number of days of hospitalization owing to the lack of a specific protocol for discharge planning in the study.

Nutritive suck, suck-swallow and swallow-respiration coordination are key components underlying the improvement of oral feeding outcomes. Knowledge on how the underlying mechanisms mediate these vital coordinative functions is very limited. Fucile et al. [42] investigated the impact of oral and particularly nonoral sensorimotor input (tactile/kinesthetic sensorimotor input to the trunk and limbs) on sucking, swallowing, and respiration. They hypothesized that multiple stimulation sites may potentially impact common underlying systems or may provide multiplicative effects on these coordinative functions. Therefore, the purpose of their study was to further explore whether preterm infants who received an oral and tactile/kinaesthetic, or combined (oral + tactile/kinaesthetic) intervention,

before the introduction of oral feeding, demonstrated more advanced nutritive sucking, suck-swallow and swallow-respiration coordination than controls. Preterm infants who received a combined (oral + tactile/kinaesthetic) intervention demonstrated more advanced nutritive sucking, suck-swallow, and swallow-respiration coordination than those who received an oral or tactile/kinaesthetic intervention singly. Results of this study demonstrated that all three interventions impacted to some degree the coordination of the above-mentioned functions related to oral feeding. The oral intervention was the only one that resulted to lead to more mature nutritive sucking skills compared to controls. These improvements may be due to the direct sensorimotor input to the oral musculoskeletal system involved in sucking [43]. It also resulted that duration of the sensorimotor intervention is an important determinant for the achievement of specific nutritive sucking skills.

In their prospective study, Lau and Smith [44] aimed to determine whether the defined oral feeding skills levels can be used as an objective tool for the assessment of preterm infants' oral feeding skills. They hypothesized that the more mature an infant's oral feeding skills level, the better his/her oral performance at that feeding; the more premature an infant, the more immature his/her oral feeding skills level, and the better the oral feeding skills levels, the faster independent oral feeding will be attained. This study demonstrated that the oral feeding skills levels were positively correlated with an infant's feeding performance, that is. the better the levels, the greater the oral performance and the shorter the feeding duration; the oral feeding skills levels positively correlated with gestational age (GA), that is, the less premature the infant, the more mature his/her skills; and the better the skills, the faster the attainment of independent oral feeding. From this study, it is proposed that the use of oral feeding skills levels can offer a more objective indicator of infants' ability to feed by mouth than GA or other tools currently available.

Mention should be made to the debate on whether there is a preferred bottle nipple to be used to enhance the bottle-feeding performance of a preterm infant. scheel et al. hypothesized that feeding performance can be improved by using the bottle nipple with the physical characteristics that enhance infants' sucking skills. A particular bottle nipple that enhanced bottle feeding in healthy VLBW infants was not identified. Based on the notion that afferent sensory feedback may allow infants to adapt to changing conditions, we speculate that infants can modify their sucking skills in order to maintain a rate of milk transfer that is appropriate with the level of suck-swallow-breathe coordination achieved at a particular time [45].

Fucile et al. showed that a controlled-flow vacuum-free bottle system versus a standard bottle facilitated overall transfer and rate of milk transfer and shortened oral feeding duration in VLBW infants. Their aim was to understand the basis by which this occurred. They speculated that oral feeding performance improved without significant change in sucking effort with a controlled-flow vacuum-free bottle system as compared to standard bottle.

In addition, they showed that VLBW infants can tolerate faster milk flow than currently presumed and that the use of a controlled-flow vacuumfree bottle system may reduce energy expenditure as it enhanced feeding performance without increasing sucking effort [46].

Conclusions

Inadequate feeding capabilities in preterm infants often lead to poor nutritional and growth failure. Although the prevalence of feeding difficulties in preterm infants is well recognized, the nature of feeding milestones including the timeline of the acquisition of the coordination of sucking, swallowing and respiration still needs more research.

This paper found that increasingly robust alertness prior to and during feeding does positively impact the infant's feeding skills especially in their ability to generate numbers of sucks and numbers of sucks per burst. There is a significant difference in the coordination of swallowing and breathing between <34 and >34 weeks' PMA; in preterm infants the maturation in respiration during feeding is not established fully yet at 36 weeks' PMA. This paper also found that oral and nonoral sensorimotor interventions provided singly or in combination shortened the transition time to independent oral feeding in preterm infants. These findings demonstrated that sensorimotor interventions have beneficial effects beyond their specific site of input. This paper underlined that preterm infants who received a combined oral and sensorimotor intervention demonstrated more advanced nutritive sucking, suck-swallow, and swallow-respiration coordination than those who received an oral or sensorimotor intervention singly. The use of oral feeding skills levels can offer a more objective indicator of infants' ability to feed by mouth than gestational age or other tools currently available. Finally, there were no studies that identified a particular bottle nipple that enhanced bottle feeding in healthy VLBW infants.

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