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Dissertation is accepted for the commencement of the degree of Doctor of Medical Sciences on May 26, 1999 by the Doctoral Committee of Medical Sciences, University of Tartu, Estonia

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Commencement: September , 1999

The publication of this dissertation is granted by the University of Tartu

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Tiigi 78, Tartu 50410
Tellimuse nr. 437

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by Roman numerals (I–V), and on some unpublished data:

I Varendi H., Porter R. H., Winberg J. Does the newborn baby find the nipple by smell? Lancet 1994; 344: 989–990.

II Varendi H., Porter R. H., Winberg J. Attractiveness of amniotic fluid odor: evidence of prenatal olfactory learning? Acta Paediatrica 1996; 85: 1223–1227.

III Varendi H., Porter R. H., Winberg J. Natural odour preferences of newborn infants change over time. Acta Paediatrica 1997; 86: 985–990.

IV Varendi H., Christensson K., Porter R. H., Winberg J. Soothing effect of amniotic fluid smell in newborn infants. Early Human Development 1998; 51: 47–55.

V Varendi H., Porter R. H. Maternal breast odor influences newborn's spontaneous prefeeding behavior. Infant Behavior and Development (submitted for publication).

ABBREVIATIONS

AF	amniotic fluid
MHC	major histocompatibility complex
VNO	vomeronasal organ
CS	conditioned stimulus
CR	conditioned response
UCS	unconditioned stimulus
UCR	unconditioned response

1. INTRODUCTION

Amongst human senses olfaction has not been considered on the equal level of importance with seeing and hearing, because olfactory impairment does not seemingly cause disability. It has been observed that (non-human) mammalian olfaction is highly involved in communication, and influences various aspects of neonatal behavior that contribute to offspring survival, such as mother-infant recognition and bonding. In mammals, several types of pheromones have been detected (Doty 1998) that regulate the behavior and neuroendocrine state of the recipient individuals: timing of weaning in rats (Leon and Moltz 1972), dominance relationships among male elephants (Rasmussen *et al.* 1994), nipple-search pheromone in rabbits (Hudson and Distel 1983). Mice avoid inbreeding by means of olfaction — they prefer mates whose body odor carries information of dissimilar major histocompatibility complex (MHC) phenotypes (Potts and Wakeland 1993).

Today, there is evidence of human ovarian-dependent pheromones (Stern and McClintock 1998), as well as capability of human females to distinguish olfactorily between persons of similar and dissimilar MHC phenotypes, showing preference for MHC-dissimilar men's odors (Wedekind *et al.* 1995). These findings confirm that the potential for chemical communication involving sexual function (and possibly parturition and childbirth as consequences of it) has been preserved in humans during evolution (Weller 1998). By examining DNA from a variety of mammals, including humans, it was determined that about 1 percent of all our genes are devoted to the detection of odors, making this the largest gene family thus far identified in mammals (Axel 1995). Among primates, humans seem to be most richly endowed with scent-producing glands and there is a growing body of evidence of strong links between the nose and human physiology and emotions (Stoddard 1991). Smell is considered in humans the most evocative of senses, underlining its central connections to cognitive centers of the brain (Tirindelli *et al.* 1998). Thus, the importance of olfactory input in human behavior has been revised during the last decade, and olfactory, behavioral and learning capabilities of the human fetus and newborn have been the objects of intensive research.

Remarkable progress in developmental neurobiology has established that although the general organization of the central nervous system is determined genetically, its fine details are influenced very much by the environment, early sensory input, experiences and relationships. As the basic problems of reproductive life are essentially the same for all mammalian species (*incl.* humans), animal research helps elucidate the evolutionary selection of the basic programs responsible for the needs and adaptive capacity of the human species in relation to child birth (Kjellmer and Winberg 1994).

2. BACKGROUND OF THE STUDY

2.1. Changing attitudes in fetal and neonatal research

The birth of a child is one of the most important milestones that marks the beginning of a completely new period in the parents' life. At the same time birth marks for the sensorily and behaviorally capable baby a transition from a prenatal to a postnatal environment. Research conducted primarily within the last two decades has provided considerable information regarding behavioral and sensory capabilities of the fetus and newborn: cutaneous senses (touch, pain, temperature), proprioception (vestibular and kinesthetic senses), vision, chemosensation (taste, olfaction) and audition.

Prior to such scientific investigations, there were a multitude of differing views ranging from one extreme that the fetus may be considered as a well-formed, well-experienced individual in his mother's environment to the other extreme where the fetus was viewed as a conglomeration of cells developing in isolation from environmental influences (Hepper 1992).

The origins of human behavior and abilities have been the subject of much fascination and speculation. Early notes about fetal sensations were offered by Aristotle who argued that the individual first acquired sensation during gestation and could thus experience various aspects of his environment. The great Indian medical scientist Susruta proposed 2500 years ago that the fetus becomes aware of his surroundings at about 12 weeks of age and actively searches for sensation. At 5 months the fetus wakes and acquires a mind, and has an intellect at 6 months (Susruta 1954, reviewed by Hepper 1992). There are numerous written speculations from the past and later history by many authors about the influence of the mother and her environment on the fetus and its development, including the explanation by Sir Kenelm Digby of King James' phobia to the sight of a naked sword as the result of influences experienced by him whilst in the womb (Graham 1951).

During the last decade new technology has allowed direct observation of the fetus "in action" and thus increased fetal accessibility. The increased interest towards the prenatal period may be attributed to a change in the views regarding the capabilities of the newborn. Earlier the newborn was regarded as a physiological organism, and research concentrated on physiological aspects of development such as growth (Rau 1982). The reason why neonatal research has changed to a new quality is not the sudden increase in neonatal abilities, but rather because scientists are now able to ask the right questions and that researchers have adopted techniques appropriate to the newborn (Hepper 1992).

2.2. Development of olfaction in the fetus

2.2.1. Anatomical development

The fetal nose develops during the first 6–8 gestational weeks and can be detected ultrasonographically at the end of the first trimester (Christ and Meinigier 1983). The nasal chemoreceptive system is composed of four subsystems: the main olfactory, the trigeminal, the vomeronasal and the terminal (Schaal *et al.* 1995-a).

From the end of the first trimester of gestation, the olfactory subsystem (the main olfactory mucosa, the main olfactory bulbs and higher olfactory centers) is mature enough to be capable of sensory performance (Pyatkina 1982). The trigeminal subsystem is one of the earliest developing sensory systems in the fetus (Humphrey 1978). It does not require specialized sensory receptors and may therefore be the earliest functioning chemosensor (Schaal *et al.* 1995-a). The trigeminal free nerve endings that innervate the nasal cavity reach *lamina propria* below the main olfactory mucosa by gestational week 18 (Chuah and Zheng 1987). The overall development of vomeronasal subsystem has been shown by the end of the first trimester (Nakashima *et al.* 1984), and its degree of differentiation is morphologically comparable to that of adult mammals at 18,5 gestational weeks (Humphrey 1940). In humans the vomeronasal organ (VNO) does not achieve maximum size during fetal development (Smith *et al.* 1997). The role of the VNO, as an organ devised to smell water-dissolved compounds, has been proven in rat fetuses only (Pedersen *et al.* 1983). Relevance of this finding to the human fetus remains unsolved. The elements — free nerve endings in the anterior part of the *septum nasi* and the main olfactory mucosa — of the terminal subsystem can be detected during the second month of fetal development (Bossy 1980), but its function has not been established yet.

Thus, by the third trimester of gestation, the fetal olfactory system is mature enough to respond to chemical stimuli (Chuah and Farbman 1995), which is supported by behavioral responses to odorants in premature infants born after 28 weeks of gestation (Sarnat 1978; Pihet *et al.* 1997). The role of each of the above-mentioned subsystems in the chemoreception process is unclear. There is conventional agreement that the olfactory subsystem is responsible for the analysis of low concentrations of volatile molecules in gaseous phase, the VNO detects nonvolatile compounds in an aqueous medium (at least in non-human mammals), while the trigeminal system is sensitive chiefly to the tactile side-effects of highly concentrated chemical stimuli (Schaal *et al.* 1995-a).

Animal studies have shown that the VNO and the main olfactory system differ in the secondary projections to the cortex and in the responses elicited by the two systems. The projections from the main olfactory system activate higher cortical centers, resulting in measured emotional or cognitive response.

Vomeronasal neurons project directly to the *amygdala* and hypothalamus (Winans and Scalia 1970; Scalia and Winans 1975). Stimulation of the vomeronasal system can coordinate the activation of central neural pathways with dramatic neuroendocrine changes to elicit a characteristic array of innate reproductive and social behaviors (Dulac and Axel 1995). The VNO detects in mammals pheromones (Wysocki and Lepri 1991) that are used for intraspecific communication. Proteins seem to act as pheromones (Mucignat-Caretta *et al.* 1995). Recently two large receptor families were identified in rats that probably mediate pheromone detection (Tirindelli *et al.* 1998). Data about development of functional details of the VNO are controversial. In the human the zones of the main olfactory epithelium appear to be defined already at the earliest stages of embryonic development but the layers of the VNO develop only during the first few postnatal weeks (Berghard and Buck 1996; Herrada and Dulac 1997). It has recently been established that there is a logarithmic volume increase for the vomeronasal epithelium and the lumen of the VNO with increasing fetal postmenstrual age (Smith *et al.* 1997). Thus, the role of these findings, mentioned above, in human fetal and neonatal olfaction remains unclear.

2.2.2. How can the odorants reach fetal olfactory receptors?

After the resorption of nasal plugs by approximately 6 months' gestation, AF begins to flow through the oral-nasal passages, thereby providing potential access to nasal receptors (Logvinenko 1990). A large amount of AF flows through the nasal passages — double the volume that the fetus swallows (Duenholter and Pritchard 1976). The respiratory movements of the fetus induce pulsatile displacements of fluid in the nasal cavity.

Secondly, the odorants can get access to the nasal chemoreceptors *via* the fetal blood (Bradley and Mistretta 1975). This might be facilitated in the fetus as the vascularisation of the main olfactory mucosa in the fetus is more pronounced than in adults (Chuah and Zheng 1987). The hematogenic pathway may be the main direct route for chemostimulants of maternal origin to the receptors. In the fetus about half of the umbilical vein blood flows directly through venous duct to the head/brain area, whereas the remaining amount of blood passes through the liver and undergoes metabolic processes there (Edelstone *et al.* 1980). The blood-borne odorants that pass through the portal tract can reach the fetal nose after several stages of metabolic processes in the liver and kidneys and excretion to the AF, that may weaken and alter their olfactory specificity and power. Olfactory stimuli in AF that flow through nose channels might therefore be qualitatively and quantitatively different from those, carried in the blood that pass the capillaries of the nasal cavity. The physiological role of the difference of the two pathways to the fetus is unclear, as well as the fetal ability to discriminate between stimuli of maternal and fetal origin. In the prenatal environment olfactory and gustatory chemosensory stimuli are not distinguished, because it is likely that both senses are stimulated (Hepper 1995).

2.3. Amniotic fluid odor

2.3.1. Composition of amniotic fluid odor

AF as a reflection of fetal and maternal metabolism has been investigated extensively. The main purposes of this research have been connected with chemical markers of fetal (lung) maturity, developmental disorders *etc.* Also there are several reports about the distribution of different drugs from the mother to the fetus and AF (Hallak and Cotton 1993). Specific odor of AF has been reported as being helpful in diagnosing maple syrup disease or trimethyl-aminuria (Lee *et al.* 1975; Menkes *et al.* 1954) and organic acidemias (Coude *et al.* 1990; Nichols *et al.* 1978). Strong odor of AF in every-day practice is considered also as a sign of amnionitis (Hauser *et al.* 1985).

There are reports of the presence of a wide range of chemical compounds in human AF. By means of chromatographic methods, alltogether *ca* 390 distinct compounds have been detected in AF at 18 weeks of gestation, whereas 120 compounds as an average have been established in individual AF samples (Antoshechkin *et al.* 1989). Amongst them a variety of potentially odorous compounds could be detected. AF analyses of different parturient women who belong to the same culture show very similar gas-chromatographical patterns of odoriferous compounds (Schaal *et al.* 1995-a).

In a recent study mothers and fathers were able to distinguish their own infant's AF from a non-related infant's AF sample by its specific odor (Schaal and Marlier 1998). They often described that their infant's AF odor reminded them of the odor of other related individuals. At the same time, the odor of AF spontaneously evoked a memory of the dominant odor of the delivery room at the moment when the infant was being expelled. The fact that individual AF samples can be olfactorily recognized by adults supports the suggestions that each fetus develops in his own unique chemosensory prenatal environment. Often the smell of AF or the baby is reminiscent of the mother's body or excretions' smell (Schaal and Marlier 1998). It is obvious that the mother and her fetus constitute an unique coexisting, two-way system — the aromatic compounds of maternal diet and metabolism pass through the placenta into the fetal body and from there into the AF. At the same time the fetus emits odorous substances into the AF and into the mother's blood that are externalized into the mother's excretions (sweat and other skin glands, urine) and compose mother's body odors (Beauchamp *et al.* 1994; 1995). It has been proven that, as in the mouse, human fetal odortypes of paternal genomic origin are represented in the odortype of the mother, doubtless by circulatory transfer of the pertinent odors (Beauchamp *et al.* 1995).

2.3.2. How do the odorous compounds reach AF?

There is no evidence that the compounds circulating in maternal blood are directly transfused to AF. They can reach AF by passing through the placenta and the fetus. These compounds can mainly be emitted to AF as consistuents of fetal urine, to a less extent with pulmonary liquid and fetal saliva. About half of the substances of maternal origin undergo metabolism by the fetus, whereas the remaining part could be excreted unchanged. Fetal urine contains several compounds that may influence the chemical environment of the fetus; for example urea may cause an ammonia-like odor (Budavari 1989).

The profiles of compounds in fetal blood or AF depend on maternal dietary intake, placental transfer and fetal metabolism. Hauser with co-authors described newborns with peculiar odors after their mothers of oriental origin had consumed cumin, fenugreek or mango, saffron and curry within a short period prior to delivery. The authors speculate that the spices were absorbed from the maternal gastrointestinal tract and transferred to the AF, where they were swallowed by the fetus (Hauser *et al.* 1985).

Transfer of flavors to AF from the meal consumed by the mother can be very rapid. When the women had ingested capsules containing the essential oil of garlic 45-min prior to amniocentesis procedure, their AF was judged to be stronger or more like garlic than were control samples (Mennella *et al.* 1995).

The placental crossing and transfer to AF of individual compounds depends on their physicochemical properties. The permeability to hydrophilic constituents is proportional to the coefficient of free diffusion in water. The food flavors, that are often highly lipophilic substances, are rapidly transferred to the fetal pool, but enter slowly into the amniotic pool (Seeds 1980). The increased placental permeability within the last weeks of pregnancy enhances the transfer of maternal dietary flavors to the fetus.

A perceivable smoky odor could be detected in the AF of smoking mothers (Schaal *et al.* 1998) as evidence that the (possibly toxic) substances inhaled by the mother influence the composition of the fetal environment.

2.4. How to observe fetal learning?

There is now little doubt that the human fetus is capable of learning. It has been investigated by means of different paradigms.

Habituation, *i.e.* the decrement in response after repeated presentation of a stimulus (Thompson and Spencer 1966), is essential for the efficient functioning and survival of the organism, enabling it to ignore familiar stimuli and attend to new stimuli (Hepper and Leader 1996). Studies have demonstrated prenatal learning as habituation of fetal body movements to a vibratory stimulus (Leader *et al.* 1982), habituation of fetal heart rate to a pure tone (Goodlin and Lowe 1974) and pink noise (Granier-Deferre *et al.* 1985). Auditory habituation has been demonstrated as early as 23 weeks of fetal age (Leader *et al.* 1984) — the age that corresponds to the onset of fetal auditory abilities. Olfaction and taste systems are functional at even earlier gestational ages, but there is no proven evidence as yet about fetal olfactory habituation.

Classical conditioning involves the pairing of an unconditioned stimulus (UCS) which elicits an unconditioned response (UCR), with the conditioned stimulus (CS), which is initially neutral and does not elicit the conditioned response (CR), but after several paired stimulations elicits CR. There are studies demonstrating conditioning of fetal behavioral responses to 500 Hz auditory tone as the CS and vibration (120 Hz) as the UCS as early as fetal age 5,5 months (Hepper and Shahidullah 1994). There is an observation of intrauterine classical conditioning using relaxation of pregnant women as the UCS paired with sound as the CS in gestational weeks 22–36. Presentation of the sound stimulus before birth reduced the latency for fetal movements, after birth these babies stopped crying, opened their eyes and showed fewer clonic movements (Feijoo 1975, 1981).

Several studies have exposed the fetus to a stimulus before birth and then observed his/her response to the same stimulus after birth. The most well observed field covers human fetal auditory learning — there are elegant studies reporting fetal and neonatal responses to the mother's voice (DeCasper and Fifer 1980; DeCasper and Spence 1986; Fifer and Moon 1989; 1994); fetal and neonatal ability to discriminate the language heard *in utero* from another language (Mehler *et al.* 1988; Moon *et al.* 1993); calming effect of maternal heartbeat on neonates (Salk 1960, 1962; DeCasper and Sigafoos 1983); fetal as well as neonatal responses to the theme tune of a popular TV soap that they had been exposed to during pregnancy (Hepper 1991).

The above studies have demonstrated successful auditory learning and the presence of memory abilities in the fetus. Fetal memory may serve a "practice function" (Hepper 1996), be important for the development of attachment and maternal recognition and the acquisition of language.

2.5. Evidence of prenatal olfactory learning

Convincing evidence of prenatal olfactory learning and memory has been documented in several mammalian species (Schaal and Orgeur 1992). These data have served as a major impetus for investigating analogous phenomena in our own species and will therefore be reviewed briefly in the following sections along with the results of recent relevant studies of human fetal/neonatal behavior.

2.5.1. Positive responses to amniotic fluid odor

Rat pups rely on olfactory cues to locate their mother's nipples. The initial orientation to the nipple by newborn pups depends upon the odor of amniotic fluid and saliva that the mother had spread on her ventrum while grooming herself during parturition (Blass and Teicher 1980). Effective sucking is disrupted after the nipple region of anesthetized lactating females is thoroughly washed (Teicher and Blass 1976). Attraction to AF has been documented also in young mice (Kodama 1990), piglets (Parfet and Gonyou 1991) and lambs (Schaal *et al.* 1995-b). Moreover, 8-hr. old rat pups, as well as pups delivered *via* caesarean section and tested immediately after birth, can distinguish their own AF from unfamiliar AF (Hepper 1987). By the start of our studies there was no proven evidence of the human newborns' responses to AF nor of human prenatal olfactory learning.

There is some evidence that human infants retain an inborn capability to recognize salient breast odor of lactating women: at two weeks of age, infants who had fed exclusively on artificial formula oriented preferentially to the breast odor of an unfamiliar nursing woman when paired with such cues from a nonparturient female or axillary odors from the same lactating female (Makin and Porter 1989). More surprisingly, when an unfamiliar mother's breast odor was paired with the scent of the formula that infants had eaten since birth, the former odor elicited more positive (head turning) responses (Porter *et al.* 1991). This phenomenon could be connected with prenatal learning of substances in AF that are present in the environment of all fetuses and are also constituents of breast odors of all lactating mothers. As discussed previously, chemical analyses have revealed similarities in the profiles of volatile metabolites found in samples of human breast milk and amniotic fluid (Stafford *et al.* 1976). Although the fetus is not exposed directly to maternal breast odor *per se*, prenatal experience might still play a role in the preferential responses to that scent observed after birth. Evidence for robust prenatal learning raises also the question: does the prenatal exposure of food flavors and alcohol influence development of preferences in later life? Moreover, early experience might play at least a facilitating role in the later expression of genetically determined behavioral tendencies like alcoholism and over-consumption of fatty foods (Hudson and Distel 1999).

2.5.2. Experimental manipulations of AF odor

Rat pups that had been exposed *in utero* to citral *via* injection of the odorant directly into the amniotic sac, and also received additional exposure to that scent following birth, selectively grasped citral-treated nipples rather than unwashed control nipples (Pedersen and Blass 1982). Such preferences for citral-treated nipples were not displayed by pups that only had postnatal contact with citral odor prior to testing. In a similar manner, rat pups that had been exposed prenatally to the taste/odor of apple juice injected into their AF subsequently showed evidence of an enhanced postnatal preference for drinking apple juice (Smotherman 1982-a).

Odors and flavors may also be introduced indirectly into the AF by adding them to the maternal diet. Twelve-day old rat pups born of mothers who consumed raw garlic while pregnant oriented preferentially to garlic rather than onion odor (Hepper 1988) in contrast to the offspring of control mothers. Similar effects of maternal diet on fetal olfactory learning and subsequent postnatal stimulus preferences have been described in lambs (Schaal *et al.* 1995-b) and rabbits (Altbacker *et al.* 1995). It was shown in rabbits that prenatal odor exposure results in a stimulus-specific enhancement in sensitivity at the level of the olfactory epithelium (Semke *et al.* 1995).

Rodent fetuses can also learn to associate an odor with a concurrent noxious or painful stimulus (odor aversion learning). This is illustrated by an experiment in which fetal rats were exposed to apple juice by injecting that liquid into the amniotic sac (Smotherman 1982-b). Immediately afterwards, the same fetuses received an intra-amniotic injection of lithium chloride, an illness-inducing substance. At the age of 10 postnatal days these pups displayed heightened avoidance of apple-juice odor, relative to control animals.

There are no data available about direct, *in utero* demonstrations of nasal chemosensation in the human fetus. Obviously, many of the above-discussed experimental treatments used with laboratory animals are not appropriate for human fetuses and infants. However, Hepper (1995) recently used an innocuous method to assess the effects of fetal exposure to a specific odorant in our own species, that was patterned after closely related experiments with rats (discussed above). One group of participating mothers consumed at least 4 meals containing garlic per week during the final month of pregnancy. Mothers in the control group never ate garlic. Babies born of the mothers who consumed garlic while pregnant displayed at their postnatal age of 15–28 hrs a slight (but non-significant) head-turning preference for the garlic odor, while the control newborns exhibited a strong avoidance of the garlic scented pad (*i.e.* oriented significantly longer to the clean stimulus). To date, this (Hepper 1995) appears to be the only report of direct, statistically reliable experimental evidence of prenatal olfactory learning and postnatal memory in humans.

2.6. Early odor-mediated behavior in mammalian neonates

There is a lot of evidence that mammalian neonates exhibit interest and attraction to odors of prenatal (AF) and postnatal (maternal) origin. Teicher and Blass (1977) showed first that AF is attractive to newborn rats, moreover, it is responsible in guiding the neonates to the nipple for the first sucking bout. Without this guidance the pups are not able to find the nipple and could not survive. The success of early postnatal nipple-search behavior, which is of life-sustaining importance for mammalian neonates, is dependent on the odor of maternal specific ventral substances (nipple-search pheromone?) in piglets (Morrow-Tesch and McClone 1990) and rabbits (Hudson and Distel 1983). The outstanding olfactory and gustatory source and a central focus of early postnatal life is breast milk. For neonates, there are several additional attractive olfactory stimuli of postnatal origin, including maternal inguinal gland secretion for lambs (Vince and Ward 1984), maternal saliva and caecotrophe — volatile substance that rat mothers secrete in the large intestine and excrete with faeces — (Galef and Muskus 1979), as well as the rat pup's own saliva (Plagge 1935). Rat pups mark the nipple during the first suckling episode with their own saliva that will be necessary for further attachments to the nipple (Teicher and Blass 1976).

Olfactory information contributes in various ways to the sucking-nursing bond in some nonhuman mammals (rats, piglets, rabbits, dogs, cats *etc.*), but does not appear to contribute to sucking behavior in farm animals born with their eyes open and capable of standing. Humans are intermediate in that they will accept a surrogate nipple but discriminate food on the basis of olfaction, and changing the familiar scent can be disruptive (Blass and Teicher 1980; Mennella and Beauchamp 1991).

2.7. Inborn behavior patterns in human newborns

Desmond (1963) called the immediate postnatal activity alerting exploratory behavior. Although the terms "alerting" and "exploratory" had been used earlier to describe animal behavior, the reactions of newborn infants were strikingly similar. Characteristic reactions and responses during the period of intense activity included:

1) nasal flaring or "sniffing" — an opening and closing of the nostrils unrelated to respiratory activity;
2) movements of the head from side to side;
3) outbursts of rapid flexor-extensor movements of upper and lower extremities;

4) sucking, chewing, rooting, chewing of fingers, protrusion of tongue;
5) outcries, abrupt onset and cessation of crying.

These findings were not investigated and considered further for almost 25 years until the studies by a group of Swedish midwives (Widström *et al.* 1987). They "rediscovered" that the human infant is provided with an inborn behavioral program that appears similar to that of other mammals. This program consists of motor activity leading the neonate to the mother's nipple and to the first suckle, provided that it is placed on the mother's chest and that its behavior is not interfered with (Widström *et al.* 1987). The newborn infants were laid skin-to-skin on the mother's chest immediately after birth. During the following hour the infants' wakefulness rose to a more alert state and they turned their heads from side to side scanning the mother's chests. The infants' sucking and rooting movements gradually increased, hand-to-mouth movement was observed and by the age of 55 minutes the infants reached the mother's nipple and started to suckle (Widström *et al.* 1987). This behavior can be depressed by pethidine that the mother receives close to delivery (Nissen *et al.* 1997).

If the infants are separated from the mother shortly after birth they exhibit crying that may correspond to the separation distress call recorded in mammals. The cry stops if the baby is reunited with the mother (Christensson *et al.* 1995).

In mammals, behaviors that place the infant in a favorable position for nipple grasping are often described as rooting (Koepke and Bigelow 1997). Nosing and probing in the mother's fur, behaviors that rat pups show just prior to nipple attachment, are referred to as rooting (Hofer *et al.* 1981). Rooting has been conceptualized as a part of the nipple attachment component of their suckling behavior (Stoloff *et al.* 1980). Nosing and nuzzling have also been observed in other mammals (Koepke and Pribram 1971). The common element among the behaviors appears to be that they put the infant's mouth and body in the proper position for elicitation of the behaviors involved in grasping the nipple (Koepke and Bigelow 1997), followed by rhythmic movements of the head, mouth and tongue that stimulate milk availability (Smotherman and Robinson 1994).

Before our studies, the factors that may elicit the above-described pre-feeding behavior and contribute in guiding the human infant to the nipple, as well as the infants' capability to display such behavior later than the first postnatal hour, had not been studied.

2.8. Early responses to odors by human newborns

Normal term newborns display differential facial expressions (believed to reflect liking, enjoyment, satisfaction or disliking) to food related olfactory stimuli at birth, prior to any feeding experience (Steiner 1977). The ability of infants to recognize their own mother is considered an important component in the development of mother-infant social relationships (Bowlby 1969). Macfarlane first (1975) tested 6–days old breast-fed infants who showed preferential orientation to their own mother's breast pad over the pad of an unfamiliar nursing woman. Moreover, there are data available indicating soothing (reduced movements of the arms and head) effects of the own mother's odor on 3-day old newborns (Schaal *et al.* 1980). Breastfed infants respond preferentially to odors from their own mother's axillary region (Cernoch and Porter 1985) and neck (Schaal 1984), but not to similar odors of unfamiliar lactating females.

Marlier and Schaal (1997) examined the ability of newborns at the age of 4 days to differentiate between the odors of their familiar food and an odorless control stimulus or an unfamiliar food. The breast-fed infants showed longer head orientation and oral activity towards their own mother's milk compared to the control stimulus or an unfamiliar mother's milk, whereas the bottle-fed newborns displayed no preference for the odor of their familiar formula over an unfamiliar formula. Two-week old, bottle-fed since birth infants are not capable of recognizing their mothers by their axillary odor (Cernoch and Porter 1985). The disparity between breast and bottle feeders is likely to be the result of differential exposure to those cues associated with the two feeding routines. Although breast-fed neonates respond preferentially to their own mother's unique scent, additional studies indicate that infants are generally attracted to breast odors produced by lactating females. Bottle-fed girls oriented preferentially to the breast odor pad of an unfamiliar nursing woman when paired with such odors from either a nonparturient female or axillary odors of the same lactating female (Makin and Porter 1989). In several studies baby girls seem to be more responsive than boys to maternal and artificial odors (Balogh and Porter 1986).

One component of breast odor is sebum, produced by sebaceal glands. The sebum excretion rate is significantly higher during the last 8 weeks of pregnancy and in breastfeeding women compared to non-pregnant controls (Burton *et al.* 1973). An experiment with rats showed that some substance(s) in the nipple area other than milk is emitted in response to circulating oxytocin and acts as an olfactory cue for the pups (Singh and Hofer 1976). The authors speculate that it is secreted from the apocrine Montgomery glands which may have the function of a scent organ.

It has been tentatively concluded that odors from lactating females may serve as two functionally distinct categories of signals for infants:

a) individually unique olfactory signatures emanating from the breast and axillary regions enable breast-fed infants to recognize their own mother;

b) general attractants associated with the breast region elicit positive responses in infants (at least female infants) regardless of whether they have had any breast feeding experience or prior contact with the stimulus female (Porter *et al.* 1992).

There are suggestions to use the natural olfactory input to improve feeding skills in the premature and high-risk newborns (Sehnal *et al.* 1989), or to place a gauze pad with the mother's milk in the infant's crib or isolette to provide pleasant olfactory input in preterm infants (Als 1982). In a recent single-subject study, the presence of maternal breast odor influenced positively the frequency of non-nutritive sucking in the preterm infant with gestational age of 32 weeks and birthweight of 1750 g (Meza *et al.* 1998).

2.9. Effects of early postnatal experience on responses to odors

During the first postnatal days infants receive an enormous amount of sensory stimuli. In repeated actions, like breastfeeding sessions, they learn to discriminate between familiar and unfamiliar input. In tests conducted in the UK by Macfarlane, 2-day old breast-fed infants did not respond preferentially to their mother's breast odor pad but at the age of 6 days and 8–10 days infants evinced a significant preference for their own mother's breast pad over the breast pad of an unfamiliar nursing female (Macfarlane 1975). Moreover, babies can recognize their own mother's axillary odor at 2 weeks of age (Cernoch and Porter 1985). In the latter study bottle-feeders showed no signs of recognizing their mother's axillary odor at the same age. This suggests that babies need a certain duration of exposure or they have to reach a certain age to be capable of postnatal olfactory learning. In a study by Schleidt and Genzel, infants were exposed to an artificial odorant that their mother applied to her breasts throughout the first 2 postnatal weeks. The one- and two-week-old infants oriented toward the familiar maternal perfume for a longer time than toward a novel odorant (Schleidt and Genzel 1990). From these results one can conclude that human infants develop a learned preference for an artificial odorant associated with the mother's breast. Balogh and Porter (1986) exposed neonates to an artificial odorant within the first 2 days after birth for approximately 24 hrs. The infant girls, but not the boys, displayed preferential orientation to the exposure odor shortly after the odorant was removed, showing very early familiarization, *i.e.* postnatal olfactory learning. In a similar study, the newborns displayed preferential orientation to that same stimulus at 2 weeks of age (Davis and Porter 1991), indicating that the newborns retain long-lasting olfactory memory from the early postnatal period.

Recent physiological research suggests that norepinephrine plays an important role in early olfactory learning (Brennan *et al.* 1990; Sullivan *et al.* 1992). Norepinephrine neurons in the *locus coeruleus* synapse with the olfactory bulb and are, at least in rats and sheep, a prerequisite for learning specific odor cues. The olfactory bulb is an important interface between receptor neurons and the brain, as well as participating directly in the memory process. At certain critical times in the life (birth?), sensitivity of the olfactory bulb might increase and its responsiveness to biologically important odors may correspondingly change (Keverne 1995). During the first hours after human birth, plasma levels of catecholamines (especially norepinephrine) are increased 20–30 fold as compared to measures from older infants (Lagercrantz and Slotkin 1986). The *locus coeruleus* is also likely to be highly activated immediately after birth (Lagercrantz 1996). Newborn babies might therefore be physiologically prepared to learn to recognize the salient (maternal) olfactory signatures and the particular olfactory stimuli to which the baby is exposed may be a significant factor in subsequent social development (Porter and Winberg 1999).

2.10. Changes in early postnatal care of newborn infants during the last decade

For a long time until the beginning of 1990-s it was a rule in former Soviet Countries that mothers and babies were separated shortly after birth. In the delivery room infants were placed under a radiant heater for the first 2 postnatal hrs, then moved to nurseries and later brought, wrapped tightly in blankets, to the mother's ward for nursing every 3,5 hrs and left for 30–50 minutes. There was almost no assistance nor advice for breastfeeding provided. The mothers could look only at the infant's face and did not have access to the baby's whole body until discharge. Prior to 1980, the first breastfeeding was recommended at the postnatal age of 24 hrs (for caesarean section babies even at 72 hrs, if at all). The nipple and the areola had to be cleaned before every single breastfeeding bout with disinfectant. The infants could not become familiar with their mother and *vice versa*. The duration of stay in maternity wards was for *primiparae* 7–8 days and for *multiparae* 6–7 days. Throughout this time the mothers could not participate in neonatal care and had to learn appropriate care themselves at home after discharge. Large amounts of infant formula were used to feed the crying infants in nurseries, mothers did not get help in breastfeeding and problems of breastfeeding were common.

In 1980-s in Tartu, A.Ormisson with her students conducted research on early breastfeeding that suggests that breastfeeding should begin within the first 2 postnatal hrs. Since 1984 in some maternity wards in Estonia the first rooming-in possibilities were established and after Estonian independence was restored and the borders opened, the routines in delivery and maternity wards have changed enormously. The aim of the care is now to encourage early mother-infant familiarization and bonding, although one still meets different (sometimes old-fashioned) attitudes and there are problems connected with separation of sick and/or preterm infants to be solved.

3. AIMS

The general aim of the study was to determine the possible influence of naturally occurring odors on the behavior of human newborns during the first postnatal days and its role in initiation of breastfeeding.

Accordingly, the present investigation had the following objectives:

1. To study the patterns of the newborn's spontaneous pre-feeding behavior (Papers I, II, III, V);
2. To determine the role of the maternal breast odors in the neonatal pre-feeding behavior (Papers I, III, IV, V);
3. To investigate the newborn infants' responses to amniotic fluid, the odor of which seems to play a great role for mother/offspring interaction in some animals (Papers II, III, IV);
4. To assess whether there are any changes in odor preferences by newborn infants during the first postnatal days and analyze the reasons of the possible changes (Papers I, II, III).

4. SUBJECTS AND METHODS

4.1. Subjects in the five studies, study conditions and the main outcome variables

The observations were performed in Tartu University Women's Clinic with healthy (weight and length appropriate for the gestational age, Apgar score >7 at 1 and 5 postnatal minutes, normal postnatal adaptation, no large malformations) full-term, vaginally delivered newborns and their mothers who had uncomplicated pregnancies and deliveries without pharmacological pain relief.

For the main characteristics of the studies see Table 1 on the next page.

4.2. Methods

All the observations carried out in Studies I, II and III had the same testing method. The naked babies were placed prone on the mother's bare chest so that they could display their spontaneous (pre-feeding) behavior and odor preferences by choosing the mother's treated breast (washed or coated with her own AF) or untreated breast (naturally smelling) for sucking.

The tests performed in Studies I, II and III resulted in two types of outcome variables:

1. sequence of spontaneous pre-feeding behavior;
2. preference for the natural or treated (coated with AF or washed) breast.

4.2.1. Observation of the newborn's pre-feeding behavior (I, II, III, V)

The "start position" for the study was the following: placing the naked baby in a prone position on the mother's chest (I, II, III), eyes at the level of the mother's nipples, nose to the midline of the mother's sternum. In Study V a warming bed surface was used instead of the mother's chest.

Observation and recording of the baby's behavior began after the baby was placed on the "start position" and continued until the baby reached the nipple and began to suck or attempted several times to grasp the nipple without success.

The following behavioral measures were obtained:

- frequency of head turns (both eyes turned to one side of the midline);

- latency for initial head turn (the first head turn by the baby after the initial placement by the observer);
- latency for the first hand-to-mouth contact (contact of the baby's fist or fingers with lips, lasting >2 sec) and its duration;
- latency for the onset of active rooting (first opening of the mouth together with slight movement of the head towards the mother's skin);
- first mouth-areola contact (first time that the baby's lips touch the mother's areola or nipple);
- sucking latency (age of the baby when nipple sucking was first observed).

Table 1. Description of the group sizes and infant ages, study conditions and the main observational measures in the five studies

Study	No of subjects tested	Postnatal age at testing	Conditions at the observation	The main observational measures
I	30	0–2 hrs	mother's one breast washed, baby on the mother's chest	preference for natural vs. washed breast; pre-feeding behavior
II	30	0–2 hrs	mother's one breast treated with AF, baby on the mother's chest	preference for natural vs. AF-treated breast; pre-feeding behavior
III				
Exp. 1	29	2–5 days	mother's one breast washed, baby on the mother's chest	preference for natural vs. washed breast; pre-feeding behavior
Exp. 2a	28	2–5 days	mother's one breast treated with AF, baby on the mother's chest	preference for natural vs. AF-treated breast; pre-feeding behavior
Exp. 2b	10 *	5–10 days	mother's one breast treated with AF, baby on the mother's chest	preference for natural vs. AF-treated breast; pre-feeding behavior
IV	47	31–90 minutes	babies separated from the mother and assigned into three odor conditions: breast smell (n=15), AF (n=16) and unexposed controls (n=16); crying recorded on tapes	crying duration
V	22	2–4 days	naked babies prone on a warming bed, tested twice: with pres-ence of the mother's breast pad or clean pad	behavioral responses to the breast pad vs. clean pad
Total no of babies studied	186			

* These 10 participated also in Exp. 2a.

Some (initial head turn, head turn, onset of active rooting) of the above-mentioned measures could be observed also in Study V.

The babies in Study I were placed on the mother's chest within the first minute after birth. In Study II the observation was started after placental detachment when the babies' age was in the range of 6–23 minutes. In Studies III and V the babies were tested at a postnatal age between 2 and 10 days.

4.2.1.1. Instructions given to the mothers during the testing procedure (I, II, III)

Prior to beginning the observations in Studies I, II and III, the mother was told that she and the observer would together watch the baby's behavior to see whether the baby could find the nipple without any help. The observer was blind to how the breasts were treated. The mothers were instructed not to tell the observer which breast had been treated and not to guide, nor help the baby with her hands. If they showed some concern about the baby's behavior they discussed it with the observer and continued to remain calm.

4.2.1.2. Testing in the maternity ward and at home (III, V)

Testing of the babies in the maternity ward occurred on their postnatal days 2–5 in Studies III and V. Additionally, in Study III, the observer visited 10 babies and mothers living in Tartu at their homes to repeat a test. Testing was performed when the baby had spontaneously woken and was presumably hungry (preferably >2 hrs since the previous breastfeeding).

4.2.1.3. Testing procedure in the warming bed study (V)

The tests were performed in the nursery using a warming bed that was provided with three 21-cm silk tapes, marked every 1 cm. The mother placed pad 1 (breast pad or clean pad, order randomized) on a piece of plastic foil on the mattress of the warming bed so that the lower and upper margin of the pad touched the 17 and 21 cm marks respectively on the central tape scale. After the pad was in place, the observer placed the naked baby prone on the warming bed with the tip of the nose on the midline, touching the 0-point of the central tape scale. At that moment, the stopwatch was switched on and the baby's behavior recorded according to the study protocol. Observations lasted for a maximum of 180 sec. The test was terminated if the baby reached the pad (forehead touched the pad and moved it from its original position) before 180 sec. had elapsed. After the first test, the baby and the test pad were removed from the warming bed. Following a 2-min pause, the mother placed test pad 2 and the second test trial was conducted as described for test 1.

4.2.2. Observation of the breast choice (I, II, III)

Placing the baby with nose to the midline as the "start position" allows the baby to sample olfactory signals from both sides of the mother's chest. During the observations the babies moved from the "start position" to one of the mother's breasts. The observer decided that the baby had made the breast choice if the baby spontaneously reached one of the mother's nipples, had mouth contact with it and spontaneously started to suck; or made several (>7) attempts to grasp the nipple without success. This baby was scored as having selected this breast for sucking; and was assisted by the observer who placed the nipple in the infant's mouth.

4.2.3. Early postnatal care of the newborn

In Study I after the cord was clamped and cut, the baby was dried and immediately laid prone skin-to-skin on the mother's abdomen and covered with a small blanket. In Studies II and IV, after the cord was clamped and cut, the baby was bathed in warm water (~37°C) with ~0.05% potassium permanganate, as routine on the ward at this time. The baby was dried and cleansed of *vernix caseosa* by using sterile sunflower oil, weighed and measured; wrapped in cotton blankets and placed under a radiant heater. Study IV continued by means of odor exposure and crying recording at the same place, whereas in Study II the baby was undressed and placed on the mother's chest at postnatal 6–23 minutes.

In Studies III and V the immediate postnatal care was not determined.

4.2.4. Preparation of the odor sources for the tests

4.2.4.1. Collection of amniotic fluid (II, III, IV)

After birth of the baby a sterile gauze pad (~7×7 cm) was moistened with the mother's own AF collected in a receptacle. If the AF (postwater) contained blood as a result of perineal rupture or episiotomia, it was not used because the blood could mask the AF odor. Instead, in these cases, AF was gently wiped off the baby's body in an area where blood or *vernix caseosa* was not seen. It was attempted to achieve similar moisture of the pads in both methods. These pads were used in the experiments (II, III, IV). For Study III, two pads moistened with AF were sealed in two different plastic bags and frozen. At the day of testing the bag containing frozen AF pad was placed in lukewarm water for thawing for ~30 minutes before the test session began.

4.2.4.2. Accumulation of breast smell on cotton (IV, V)

In Study IV, a cotton cloth (30×15 cm) was taped directly over one side of the mother's bare chest so that it covered one breast including the areola region (but not the nipple) and the corresponding axillary region. It was fastened at least 2 hrs prior to delivery, then removed after birth and presented to the baby.

In Study V, on the morning of the test day, the mother was asked to fasten a round cotton pad above the nipple on each of her breasts for at least 2 hrs. The pads were removed shortly before the testing began.

4.2.4.3. Presentation of odor to the newborn (IV)

The cotton cloth carrying mother's breast smell or gauze pad moistened with AF was attached to cover the muzzle of a plastic funnel (~8×8 cm) that was placed approximately 2-cm from the baby's nose. The cloth/funnel remained in this secure position throughout the crying registration period so that it never came into direct physical contact with the baby.

Use of the breast pads in the warming bed (V) is described in 4.2.1.3.

4.2.5. Breast treatment

4.2.5.1. Washing of the mother's breast (I, III)

To remove the natural odor, one of the mother's breasts was washed for about 15 cm around the nipple with an odorless liquid soap. The washed breast was rinsed with lukewarm water and dried.

4.2.5.2. Observation of the effect of washing on breast temperature (I)

Six additional mothers underwent similar washing procedure within 12 hours after delivery. Breast surface temperatures were recorded (electrode C 1800K, Anritsu, Japan; thermometer 52 K/J, Fluke, Litho, USA) at the nipple, border between the areola and white skin, and midway between the areola and sternum at 5–10, 15–20, 30–35 and 50–55 min. after washing.

4.2.5.3. Treating of the mother's breast with AF (II, III)

The nipple and areola zone (in a diameter of ~3 cm) of one breast was gently wiped with a gauze pad moistened with AF. The alternative breast remained untreated and neither breast was cleaned with water or other solutions.

4.2.6. Recording of infant crying (IV)

Within 16–30 min. after birth, a portable stereo cassette-recorder was placed ~20 cm from the baby's head and switched on to register audible crying. Babies stayed in the same position until the end of the observation period (*i.e.* postnatal 90 minutes). At that time, the axillary temperature was recorded and the baby was given to the mother for the first time for the initial breastfeeding session. The mother's name and age of the baby at the start of recording were recorded on the tape and the name and date were also written on the tape cassette and protocol log.

4.2.6.1. Analyses of taped cries

The final analysis of all tapes was done by a single assistant who was not aware of the condition to which individual babies had been assigned. Each baby's total crying duration was measured in seconds per standardized 15-minute period relative to the time of birth (*e.g.* 31–45 postnatal min.; 46–60 postnatal min., *etc.*). The analyses were done "by hand" with 2 stopwatches; one for measuring 15-min. periods, another for recording crying duration.

Before the analyses of the tapes began, crying was defined so that it could be distinguished from other briefer, quieter noises, such as whimpering. Measurement reliability was assessed by comparing crying duration judgements between two investigators — observer and assistant — (inter-rater reliability) and across two repeated judgements by the single assistant (intra-rater reliability) for five 5-min. periods during which a baby cried. No significant differences were found for these crying-duration comparisons.

4.2.7. Randomization to breast treatment groups and study groups

In Studies I, II and III, the mothers were assigned by closed envelope method to the two groups: treatment of the left or the right breast. Equal numbers of left and right breasts were treated. The treatment procedure was performed by an assistant, while the observer was out of the room and therefore unaware of which breast had been treated.

In Study V the mothers assisted at the experiments and placed the test pads onto the warming bed surface according to the written randomization procedure. Each baby was tested two times; once with a soiled breast pad and once with a control pad, in a randomized order.

In Study IV allocation to groups was random except for the following circumstances: when a baby was initially assigned to Condition B, but delivery occurred less than 2 hrs after the stimulus cloth was taped to the mother's breast, that infant was reassigned to Condition AF if clean AF was available, or Condition C if clean AF was not available.

4.2.8. Collection of the data concerning the postnatal care in the maternity ward (III, V)

The mothers kept a diary during their stay in the maternity ward from the birth of the baby until the testing procedure on postpartum day 2–5. They were asked to record the time at the beginning and end of the activities concerning breastfeeding and care of the baby.

They also provided information concerning the use of perfumes, deodorants, shampoos, soaps or other scented products, as well as painful or sore nipples and the application of ointments to the breasts or nipples.

The coded questionnaires were analyzed separately from the results of the tests with the babies. The number of breastfeeding bouts, total duration of breastfeeding and the cumulative duration of close contact with the mother were calculated for the total postnatal time period preceding testing.

4.2.9. Statistical methods

For the comparisons between the groups of babies displaying different breast preferences, Fisher's exact, chi-square and binomial tests were used. Because the data did not follow the normal (Gaussian) distribution, the Mann-Whitney U-test was used for the paired comparisons of the details of pre-feeding behavior and crying duration. For comparisons of crying duration between three treatment conditions the Kruskall-Wallis test was used.

4.2.10. Ethical considerations

The observations were performed after mother's (and father's if present) oral informed consent was obtained. All the studies were approved by the Ethics Committee of the Faculty of Medicine, University of Tartu.

The tests for Papers I, II and III could be assessed from an ethical point of view as very suitable for the baby and mother — early close body contact is the first step to successful mother-infant bonding. The only question that should be discussed is breast treatment. Washing and cleaning with disinfectants before breastfeeding the breast was very common and even demanded in the Soviet Union and that routine was not completely stopped by the beginning of the study in Estonia. The women believed that the uncleaned "dirty" breast should not be given to the baby. Breast treatment with AF that is not spoiled could also not be unethical as it belongs to the baby's own prenatal environment.

Separation of the mother and baby for recording of crying was at the time of the study justified because in Estonian maternity hospitals it is a very common routine even today, although the duration of separation is individual and almost all the babies are given to the mothers for breastfeeding before 60 minutes of postnatal age. In our study the mothers had given their informed consent to participate up to 90 min. of baby's postnatal life and in 5 cases (of 60 total observations) the recording was interrupted before the end of the observation period because the mothers decided to breastfeed and calm their crying babies.

5. RESULTS AND DISCUSSION

The most important results are presented and discussed in this chapter. The detailed description of the results can be found in the attached publications.

5.1. The role of maternal natural breast odors in guiding the newborn to the nipple (I, III Exp. 1, V)

We have investigated the human newborn's olfactory-mediated behavior shortly after birth (within the first two postnatal hours) and on postnatal days 3–4 in a biologically important situation — the baby was placed prone, skin-to-skin on the mother's chest, free to move and display preferences for the naturally smelling breast or for the breast, whose natural odor was removed by means of washing.

The babies of both age groups showed preferences for the naturally smelling breast, independent of the side (left or right breast), Figure 1, and we concluded that the babies responded to differences in smell. The attractiveness of maternal breast odors to the newborn is further supported by our results of greatly increased forward movement in the direction of a maternal breast pad that was placed on the surface of a warming bed 15-cm in front of the baby (V).

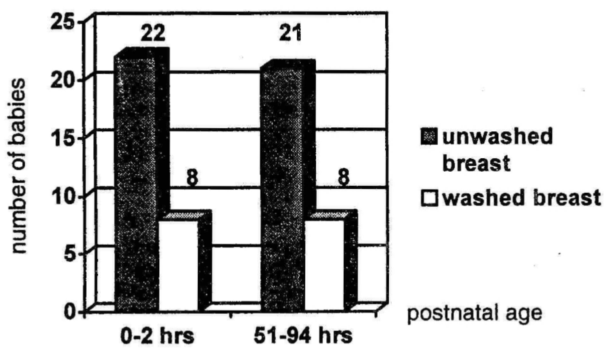

Figure 1. Number of babies who selected the naturally smelling (unwashed) breast or the washed breast for sucking within the first 0–2 postnatal hours (I) and postnatal days 3–4 (III, Exp.1).
The differences are statistically significant ($p<.03$).

These findings are consistent with earlier simple 2-trial absolute choice tests in which breast-fed infants oriented preferentially towards a gauze pad that had been worn on the breast of a lactating woman compared with an odorless control pad (Macfarlane 1975). Furthermore, exclusively bottle-fed girls could distinguish between an unfamiliar lactating woman's breast smell *vs.* axillary odors from that same woman or an unfamiliar nonlactating woman (Makin and Porter 1989). The phenomenon that baby-girls could have better olfactory discrimination ability was similarly noted in Study III (Exp. 1), but not in our remaining experiments. Our results are consistent also with studies of non-human mammals showing that washing the lactating mother's nipples/ventrum resulted in disturbed nipple finding and feeding by rat pups (Teicher and Blass 1976). The odor of maternal breast secretions may be attractive to neonates, at least in part, because of its discernible resemblance to the familiar amniotic fluid odor (see 5.2).

From a biological and practical point of view, the naturally occurring odors seem to contribute to early nipple attachment and sucking. Sore nipples, the frequent complication of breastfeeding, seem to be due to wrong attachment to the breast. The latter may be connected with inadequate opening of the baby's mouth (Woolridge 1986; Righard and Alade 1992) in the absence of stimulating odor, whereas it was shown that the infant's own mother's odor elicited increased mouthing (Sullivan and Toubas 1998). It is possible that olfactory cues contribute to a proper attachment and sucking technique. Failures of breastfeeding often remain unknown, but the early suckling pattern is of prognostic value for the duration and success of breastfeeding (Righard and Alade 1992) and the delivery ward routines are very important for a good start (Righard and Alade 1990). Cleaning of the breasts, use of scented disinfectants in the neonatal care unit and odorants by mothers and staff may mask or override the natural odors that are essential for recognition of the mother and her smell, as well as for successful breastfeeding.

We have met several similar situations during our pilot studies: in one instance, when the newborn showed no interest towards the breast even if placed very close to it, the mother recounted that she had taken a shower within the last hour (our unpublished observations). The newborn's ignoring behavior indicated that the mother's breasts did not carry any smell to stimulate the baby.

Breast washing did not have a significant effect on the breast temperature in our study. It has been shown in rats that alteration of only olfactory properties of the rat mother's nipple region, but not thermal or tactile stimuli, resulted in nipple attachment failure by rat pups (Blass *et al.* 1977; Bruno *et al.* 1980). Spiny mouse pups, whose olfaction was experimentally impaired (nasal passages irrigated with zinc sulfate), developed growth retardation and behavioral deficits, that may reflect problems with nipple finding and grasping (Porter *et al.* 1987). In the latter study, the olfactory-impaired pups developed better when kept in the same cage with their untreated healthy littermates who provided them with some visual and tactile guidance.

In former times the overall recommendations demanded routine cleaning of the breast before every (*incl.* the first) breastfeeding bout with a disinfectant that would eliminate the smell and give a strange taste to the breast. Together with late (at ~12–24 postnatal hrs) introduction of breastfeeding, strict (every 3,5 hrs, 6 times/day) regimen of feeding times, separation of mother and baby within the first postnatal hours and during their stay (6–8 days) in the maternity ward, this could contribute to failures in breastfeeding and mother-infant bonding.

5.2. Attractiveness of amniotic fluid odor (II, III (Exp. 2a and 2b), IV)

Significantly more newborns selected an AF-coated breast, rather than the untreated alternative breast, for sucking during their first sucking attempt within the first postnatal hour (II, Figure 2). This indicates that AF odor is attractive to the newborn infants and overrides or potentiates the natural smell of the maternal breast. Positive responsiveness to AF shortly after birth may be a reflection of prenatal experience and familiarization with that substance. The importance of the prenatal acquisition of olfactory information for early postnatal behavior has been proven in rats, whose pups rely on AF that the mother deposits on her nipples. Without this guidance the pups are not capable of locating the nipple and will not survive (Teicher and Blass 1977; Blass and Teicher 1980).

Although there are some studies since 1995 reporting responses of 2–4-days-old human newborns to AF (Schaal *et al.* 1995-c; Schaal *et al.* 1998; Marlier *et al.* 1998-a; Marlier *et al.* 1998-b), our results indicate that the human newborn is capable of using prenatal olfactory memory during early adaptive postnatal behavior and odor preferences shortly after birth.

We must argue with the statements of Schaal *et al.* (1998) who speculated that in our study the positive responses might be due to a combination of oral and nasal chemoreception because the newborns were in direct contact with the odor source and could lick it. In our study groups the newborns were initially placed on the mother's chest and therefore could only lick the midline area of the chest — but not the odor source — before choosing the breast. AF was applied only on the nipple and areola area, licking of which could occur only after the baby had made its decision by using nasal chemoreception and had crawled towards the nipple.

5.2.1. Changes in odor preferences during the first postnatal days

We performed a new series of tests with the newborns of postnatal age between 44–105 hours. This is the time when the newborns have successfully adapted to extrauterine life and experienced different sensory input (*incl.* the mother's extrauterine environment), also effective breastfeeding has been established. At that time we did not find significant differences in the odor preferences for AF versus natural breasts (Figure 2: III, Exp. 2a). When these infants were tested again about 4 days later, they had changed their preference from the AF-coated breast to the naturally smelling breast (Figure 2: III, Exp. 2b). These results are consistent with those of recent choice-tests performed by Marlier *et al.* (1998-a). They observed breast-fed infants' responses to AF and colostrum 22–55 hrs after birth and found no differences, but a few days later the newborns oriented significantly longer to a pad bearing their mother's transitional milk. In addition, bottle-fed infants expressed significantly longer orientation responses toward AF odor than towards the odor of their familiar formula at the age of 2 and 4 days (Marlier *et al.* 1998-b).

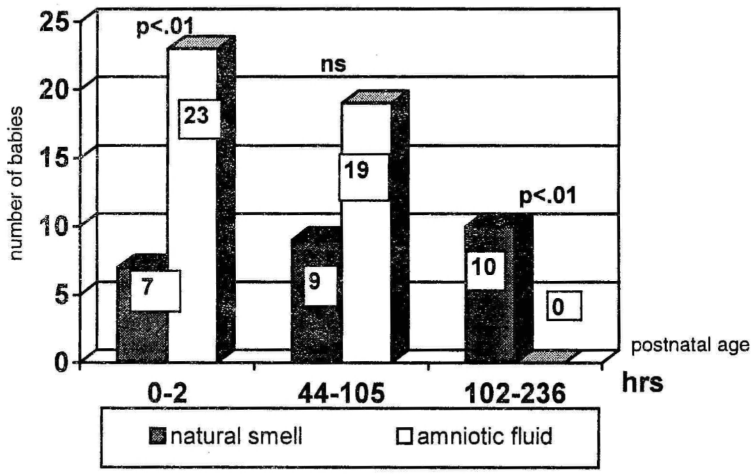

Figure 2. Number of babies who selected the AF-coated breast or the untreated, naturally smelling breast for sucking within the first 0–2 postnatal hours (II); postnatal days 2–5 (III, Exp. 2a) and postnatal days 5–10 (III, Exp. 2b).

Why do the preferences change? In a study of fetal auditory learning the evidence of prenatal learning disappeared by 21 days of postnatal age (Hepper 1991). It is obvious that prenatal memory persists for several days postnatally — the bottle-fed newborns in the above study preferred their own AF to the scent of their familiar formula even though they had not been exposed to breast milk and colostrum that could carry olfactory cues that are similar to those found in AF because constituents of the mother's diet can flavor all of these fluids. They had experienced the odor of familiar formula during several bottle-feedings, but obviously only by means of retronasal route of olfaction and this exposure was not sufficient to evoke a preference.

In the above-mentioned studies by Marlier and co-authors the choice-influencing factors that were discussed and considered were age, sex, feeding type and head-turning side. We have also recorded and analyzed the details of the early postnatal care and breastfeeding and found that the preference for the naturally smelling breast was positively correlated with the duration of the breastfeedings and close mother-infant contact before testing in these babies (Table 2). Our data provide additional evidence that intensive olfactory postnatal learning takes place within the first days. At the same time, age of the newborn could also play a role in the observed preferences — increased experience is positively correlated with age. Thus, the increased experience with maternal external odors (*incl.* breast odors) and decreasing (absent) exposure to prenatal compounds could influence the newborn's choices between AF and breast odor. Although AF odor appears to bear some resemblance to maternal breast odor, it should be mentioned that one reason for the preference changes might be that colostrum and mature milk could have dissimilar odors, which is not surprising because of their quite different composition (Neville 1995). Additionally, the gustatory and postprandial satiation (*i.e.* release of opioids) effects of breast milk could contribute to the changes in odor preferences.

Table 2. Results of choice tests on postnatal days 2–5 in Study III: Exp. 1 (the unwashed *versus* washed breast) and Exp. 2a (AF-coated *versus* untreated, naturally smelling breast), and characteristics of babies in each response category.
Data expressed as medians (range).

	Experiment 1		Experiment 2a	
	Breast choice		Breast choice	
	untreated n = 21	washed n = 8	untreated n = 9	AF-treated n = 19
girls / boys	11 / 10	0 / 8*	5 / 4	8 / 11
no of breastfeedings x	17 (6–33)	16 (8–32)	26 (14–36)	19 (13–45)
cumulative breastfeeding time, min. x	310 (105–560)	330 (90–560)	490* (235–820)	375 (200–605)
duration of mother- infant close contact, hrs x	23 (8–70)	31 (16–50)	38,5* (24–66)	25 (9–51)
age at experiment, hrs	68 (55–74)	59 (51–94)	74 (50–92)	69 (44–105)
time to first mouth-nipple contact, min.	2 (0,5–8,5)	1,5 (0,8–13)	2,5 (0,5–13)	2 (0,2–10)
treated (washed or AF) left / right breast	12 L / 9 R	3 L / 5 R	7 L / 2 R	7 L / 12 R
choice of left / right breast	9 L / 12 R	3 L / 5 R	2 L / 7 R	7 L / 12 R

* p<.05; x — prior to testing.

There could be one more explanation for the changes in preferences. During pregnancy the mother's body (and AF) odor consists of her own and her husband's odortypes (Beauchamp *et al.* 1995), but the odortype of paternal origin disappears from the mother's odor soon after delivery. Thus — the newborn might initially recognize the familiar mixture of the mother's and father's odors in both AF and colostrum, but later switches over to the mother's cues as essential markers of food source and caregiving.

Thus, familiarization with the odor of AF *in utero* might facilitate the early development of olfactory recognition of the mother. Because individual recognition is a necessary precursor of the development of specific social attachments, it could also contribute to the development of infant-mother bonding (Hepper 1987; Porter 1991; 1994; Robinson and Smotherman 1991).

5.2.2. Effect of AF on hand-mouth contact

Very few hand-mouth contacts were recorded in Study II compared to Study I (Figure 3). There were also some qualitative, although unquantified differences between the behaviors of the two groups. Thus, in Study II the babies did not seem interested in joining hand to mouth even if they were close to each other. Short-time finger sucking was elicited only if the hand happened to touch the mouth. In tests with older (20–182 hrs) newborns Butterworth and Hopkins (1988) similarly noted that once the hand touched the mouth it was withdrawn and there was little evidence of sucking the fingers.

We observed very few hand-mouth contacts and finger suckings in Studies III and V (Table 3, Figure 3), but if the baby happened to suck a finger, then the following patterns of pre-feeding behavior were delayed (unpublished observations), indicating a self-calming function of finger sucking for newborns (Feldman and Brody 1978). Hand-mouth contacts have been observed prenatally beginning at 12 weeks of gestational age (De Vries *et al.* 1984) and they seem to be a goal-directed behavior in newborns (Butterworth and Hopkins 1988).

In Study I, in contrast, we observed that if the hand was close to the nares, *i.e.* 3–4 cm, it was soon put into the mouth and finger sucking began. It seemed like the hands were not attractive for sucking in Study II, probably because bathing removed AF from the body and hands. This could support the idea that the hand-mouth contact could be enhanced by olfactory detection of AF on the skin of the hand that is attractive to the newborn baby. In studies by A.-M. Widström *et al.* (1987) and Righard and Alade (1990) pre-feeding behavior and hand-mouth contacts occurred as often as in our Study I. In these studies the babies were not washed nor bathed before observations. The occurrence of hand-mouth contact and finger sucking in unwashed newborns within the first postnatal hour might be a key behavior that contributes to the maturation and development of the child's digestive tract (Widström *et al.* 1987).

Table 3. Comparison of pre-feeding behavior during the first postnatal 90 minutes (I) and on postnatal days 2–4: on the mother's chest (III, Exp. 1) and in the warming bed with the presence of maternal breast pad (V).
Latency measures calculated from the beginning of the observation.

Number of infants tested	Postnatal 90 minutes (I)	Postnatal days 3–4 (III)	Postnatal days 2–4, warming bed (V)
	n = 30	n = 29	n = 22
onset of active rooting, min.			
— median	20***	0,17	0,1
— range	10–41	0,08–3	0,05–3
hand - mouth contact, min.	(n = 27)***	(n = 8)	(n= 8)
— median	23***	0,5	3,0
— range	7–60	0,2–1	0,1–3
first mouth - nipple contact, min.			(n=18)§
— median	52***	2	2§
— range	22–100	0,5–13	0,75–3
number of head turns	(n = 28)	(n = 18)	(n = 4)
(*excl.* the first turn)			
— median	1	1	0
— range	0–6	0–3	0–1

*** p <.001, comparison of Studies I and III; § nose contact with the maternal breast pad.

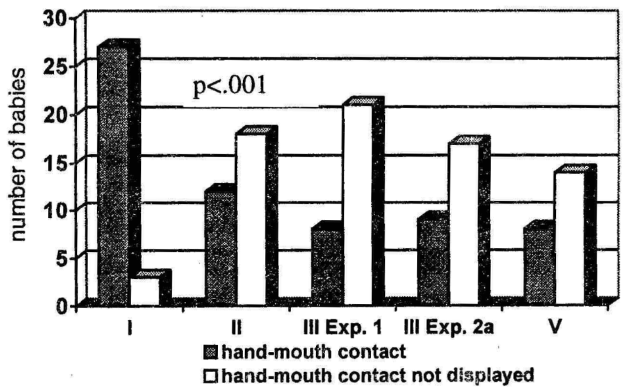

Figure 3. Proportion of babies displaying hand-mouth contacts in Studies I, II, III and V. In Study I the newborns were unwashed, in Study II washed. The remaining studies (III, V) were performed with 2–5 days-old newborns.

5.3. Pre-feeding behavior (I, II, III, V)

The awake, presumably hungry infants on postnatal days 2–10 if placed prone on the mother's chest, or onto the surface of the warming bed, displayed soon afterwards typical pre-feeding behavior. This is similar to the pattern that has been described shortly after birth (Widström *et al.* 1987; our publications I, II) but the latter is characterized by a delay of 10–40-minutes and by markedly lower speed (Table 3). The pre-feeding behavior consists of rooting movements (mouthing, lifting and movement of the head to sides), hand-mouth movement (and finger sucking, see 5.2.2 for discussion) as well as pushing with legs of variable intensity and power, that can result in movement from the initial position towards the nipple or maternal breast pad. The rooting of human infants resembles nosing and probing movements in other mammals. Rooting behavior is of importance for the infant's ability to attach itself to the nipple (Nissen *et al.* 1997) and especially for mouth opening and positioning of the tongue before attaching to the nipple (Widström and Tingström-Paulsson 1993). Lifting and side movements of the head might be helpful for sampling odors. Pushing with the legs followed by movement from the initial position resembling crawling (the upper body is very seldom involved in crawling and the hands seem quite useless in this behavior) are important in approaching the goal (*i.e.* nipple or maternal breast pad).

These activities seem to use the congenital primitive responses, which the physician examines during the neurological examination of the newborn. The "defense" response lifts the head and turns it to the side, the "search" response together with the sucking response participate in rooting if something touches the newborn's cheek or mouth, "crawling" response helps the body to move on. Crawling consists of two components — intermittent pushing (resembling stepping movements while the baby is held vertically) and intensive pushing with straightening of the whole trunk.

Absence or decrease of these, above-mentioned responses is often considered as a sign of neurological impairment (disability). From an evolutionary point of view, it may mean that the newborn is not capable of reaching the nipple, cannot get food and will not survive. It happens often to weaker mammalian neonates.

5.3.1. Differences in tempo of pre-feeding behavior

Is the origin of pre-feeding behavior genetically based or pre- or postnatally learned? The hypothesis about the involvement of genetical information is supported by the similarity of human newborn's behavior to that of other mammalian neonates. Another example suggesting that the human newborn has an innate perceptual mechanism is the ability to detect and respond specifically to faces (Walton *et al.* 1997). This capability is not prenatally learned, as infants are born visually naive, and cannot be learned postnatally, because it operates shortly after birth (Goren *et al.* 1975). Neither could the accelerated tempo of pre-feeding behavior after the second postnatal day be the result of postnatal learning: the infants whom we tested in Studies III and V, had not experienced the whole sequence of spontaneous pre-feeding behavior before the first testing on postnatal day 2–5, but performed equally well and with advanced tempo (III), compared to the babies' activity during the first postnatal hour (I, II), Table 3.

An additional reason for such differences in tempo could be hunger — the infants in Study III were tested after their spontaneous arousal for expected breastfeeding. At the same time, hunger behavior is correlated with the low pre-prandial concentration of cholecystokinin that is believed to be responsible for neonatal satiety after breastfeeding (Uvnäs-Moberg and Winberg 1989; Marchini and Linden 1992; Uvnäs-Moberg *et al.* 1993). Thirst is a regulating factor that influences intervals between two subsequent feedings in on-demand breastfeeding newborns (Marchini and Stock 1997) and could also contribute to accelerated pre-feeding behavior on postnatal days 2–5 when the physiological body weight reduction with high serum sodium and osmolality levels occurs. It is clear that the neuroendocrine axis, which aims to preserve nutritional homeostasis, is functional in newborns at birth (Marchini *et al.* 1998).

The delay of pre-feeding behavior shortly after birth (I, II) could be explained as possible postnatal exhaustion. Also, because of sufficient congenital fund of energy the newborn can concentrate on early adaptation and first sensory postnatal input for several minutes. Clinical data indicate that at the age of 0,5 hrs plasma glucose concentration decreases and it is associated with an awake state and sucking movements in newborns (Marchini *et al.* 1993). At that time the newborns who are placed on the mother's chest, display rooting and pushing with their legs and try to reach the nipple. It was shown that decreases in the blood glucose level are important for the initiation of feeding behavior in rats (Louis-Sylvestre and Le Magnen 1980).

Aside from the differences in tempo between the two age groups, there were remarkable inter-individual differences in the group observed during their postnatal 0–2 hrs. The large range of behavioral latencies (Table 3) may indicate that as early as within the first hours of life, the main features of personal temperament and character can be observed. However, the different timing of behavior might also be a result of birth stress and sympathoadrenal system activity level, that is of crucial importance for early adaptation and arousal (Lagercrantz 1996), whereas the normal variations in catecholamine levels are considerable (Lagercrantz 1994).

5.3.2. Position of the newborn and pre-feeding behavior

Some details of pre-feeding behavior, like rooting and head turning, were similar in Studies I, II and III (naked baby on the mother's chest) and Study V (naked baby in the warming bed) suggesting that they may have been elicited by the prone position and hunger rather than odors or other external stimuli.

The position of the newborn influences the behavior. It was noticed that wakefulness is more related to the supine position (Amemiya *et al.* 1991) and in the group of babies who had been nursed in the prone position, more wakefulness and crying was registered in the supine position (Brackbill *et al.* 1973), that may indicate dyscomfort. Almost all mammalian pups act normally in a prone position and may feel very uncomfortable while placed supine. Human infants have been cared for hundreds of years while supine, as seen on paintings, but have evolved better skills (*i.e.* pre-feeding behavior) in a prone position similarly to other mammals. Although in the supine position they are capable of turning the head side-to-side and make several types of movements with limbs (Desmond 1963), among these the only goal-directed and coordinated movement is hand-mouth contact that is highly organized and occurs more frequently before feeding (Feldman and Brody 1978).

5.3.3. Head turning

Schaal *et al.* (1998) described that infants exhibited an average range of 4,5 to 8,9 bilateral head movements during odor choice tests and tended thus to actually explore the olfactory scene created by the experimental setting. In our Studies I and II (postnatal hrs 0–2), where the infant's position differed from the position Schaal *et al.* used (half-seated, supine), the infants also made at least one head turn from the start position (prone, nose to the mother's chest). In 49/60 newborns we recorded additional head turnings (mean 2,4; range 1–16), indicating that the newborns sampled repeatedly the olfactory signals from both sides of the mother's chest. Several lateral head movements in response to olfactory differences between the mother's breasts (and in choice-tests) most likely reflect infants' active sampling of those two odor sources. As described several years ago, flaring of the *alae nasae* was present in the majority of infants during the first 15 postnatal minutes, and was often associated with side-to-side movements of the head so that the infant appeared to be "sniffing" (Desmond *et al.* 1963).

Additional head turning was not so often recorded in tests that were performed by us on postnatal days 2–10 — 35/67 newborns turned their heads only once before selecting the breast for sucking, Table 3 (III) — during the initial placement they could get sufficient information about the odor sources.

In Study V, moreover, where there was either no olfactory stimulus, or the breast odor was presented in front of the baby, more than one lateral head turn was recorded in only 9 of the 44 trials. The explanation could be that when the salient breast odors are positioned in front of the infant, lateral head movements do not facilitate perception or localization of the olfactory cues.

5.3.4. Failures in spontaneous pre-feeding behavior and nipple grasping

A brief period of separation of the infant from the mother during the first postnatal hour had a strong effect on the success of the first breast-feed (Righard and Alade 1990), if the interruption of immediate postnatal skin-to-skin contact was performed at about 20 minutes — the time when infants often start to display rooting and soon afterwards reach the breast and suckle. Interruption of the spontaneous pre-feeding behavior might have sequelae like failure of sucking as described by the above-mentioned authors. De Chateau and Wiberg (1984) showed that uninterrupted early skin-to-skin contact seemed to prolong the duration of breastfeeding and have a positive influence on maternal behavior. Separation in our Study II differed from the above-mentioned study: we separated the infant at birth and placed it on the mother's abdomen on postnatal 6–23 minutes when it could display spontaneous uninterrupted pre-feeding behavior.

We have also noticed several cases where babies needed assistance for grasping the nipple not connected with separation in Studies I, II and III. These some babies may have failed because of an uncomfortable position (prone); as well as inappropriate position of the mother's breasts (particularly large, soft breast or flat nipple) if she is lying on her back. This can be explained by the finding that the suckling attempt involves first pulling slightly back from the nipple and then moving toward it, usually with open mouth (Koepke and Bigelow 1997), ready to grasp it. If the breast is large and soft, head lifting might not be easy and non-shaped nipples cannot be grasped.

5.3.5. Influence of breast odors on pre-feeding behavior and orientation to the breast (Papers I–V)

When the 2–5-day old babies were tested in the warming bed (V) and a stimulus pad was presented 15 cm in front of the baby, forward movement in the direction of the maternal breast pad was greatly increased (and with a shorter latency) compared to a control pad. Significantly more babies reached the maternal breast pad than the control pad, and the median distance covered during the 180-sec. test session was greater in response to the breast pad.

Movement towards the breast pad was preceded by a stereotyped sequence of behavior similar to that described above (5.3) including pushing with the legs and crawling movements. It therefore appears that breast odors — in the absence of additional maternal cues — accelerate the sequence of pre-feeding behavior and are sufficient to guide the baby to the odor source. This physical attraction to maternal breast odor corroborates the results of earlier related studies showing that babies preferentially turned their head in the direction of a pad bearing the odor of their mother's breast (Macfarlane 1975; Makin and Porter 1989).

Our results are further evidence that odors emanating from the mother's breasts are attractive to the newborns and can elicit the complete repertoire of pre-feeding behavior, even in the absence of other maternal cues, in infants who have awaken spontaneously for breastfeeding. In any event, these data illustrate the complex influence of olfactory cues on neonatal behavior.

5.4. Effect of perinatal smell exposure on crying behavior

Isolated, wet, or distressed infants cry. Crying is an effective means of obtaining adults' attention and care in all mammal species studied, including humans. Our results indicated that crying of newborns who are separated from their mother within the first two postnatal hours can be reduced by exposing them to their own AF odor (Figure 4). This is consistent with the hypothesis that the fetus may become familiar with chemical cues present in the intrauterine environment. The newborn infant might retain a memory trace of such prenatal olfactory experience and therefore respond discriminatively to those same cues during the early postnatal period (Hepper 1995). Such recognition may help the baby to stay calm and feel a relative comfort in the absence of other maternal cues. The observed influence of AF odor on infants' crying is similar to the recently reported diminution of cries resulting from bodily contact with the mother (Christensson *et al.* 1995). However, the suppression of crying in the latter study could be mediated by a combination of maternal auditory, tactile and olfactory stimuli.

Interestingly — the newborns seemed to cry more if they were exposed to the odor of their mother's breast. Although not reliably different, the median crying durations for infants exposed to maternal breast odor were more than two times greater than that for the control babies for the total observation time and across all four 15-min. time blocks (Figure 4). In earlier studies it was stated that distress cries by human babies separated from their mother at birth lessen abruptly when maternal bodily contact is reinstated (Christensson *et al.* 1995; Michelsson *et al.* 1996). In a recent study 1–2 day old crying newborns responded to the night gown of their own mother or unfamiliar lactating woman with reduced crying and increasing mouthing movements (Sullivan and Toubas 1998).

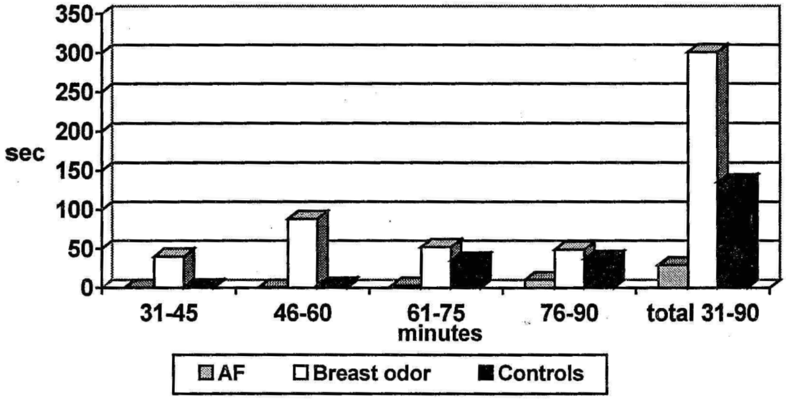

Figure 4. Median crying times (sec) for each of the four 15-min. periods and the whole 60-min. observation period in newborns exposed to odors of AF, mother's breast and unexposed controls (IV).

Why do our results show the opposite effect of the maternal breast odors? The first difference might be the age of the studied babies. Our newborns cried most intensively during the second half of the first postnatal hour — this is concurrent with the time when the blood sugar drops (Marchini *et al.* 1993) and the newborns might first feel hunger. At this time they display the typical sequence of pre-feeding behavior that ends with sucking of the nipple that is guided by the olfactory cues emanating from the mother's breast (I, II). The second concern is the duration of observation and odor exposure. In the study by Sullivan and Toubas the exposure to the mother's gown lasted for one minute. Such short odor presentation elicited increased mouthing, meaning that the newborns' brain might expect to get food or possibly to suck. In our study babies may likewise have been prepared initially to suck in response to the maternal breast odor, but they were not allowed to do so during the relatively long period of continual exposure to that odor cue and they therefore displayed heightened arousal with crying. Macfarlane (1975) had similarly noted that 2–10 day-old breastfed infants displayed increasing signs of frustration (heightened activity and crying) after their nose was in contact with a soiled breast pad for an "extended time". It therefore appears that maternal breast odors may signal the presence of an appropriate food source.

It is obvious that crying of newborns could be influenced by maternal stimuli other than olfactory cues, but one should consider also the details of postnatal care. Control babies in the present experiment cried considerably less than observed in a group of neonates of the same age tested in Madrid (Christensson *et al.* 1995). Unlike the babies in Spain, those in Estonia were swaddled and placed under a radiant heater (resulting also in a slightly higher mean axillary temperature) that could provide them with additional comfort.

5.5. General discussion

Development of perinatal and neonatal medicine has been very rapid within the last decade. Nevertheless, implementation of new technology and equipment in diagnosis, treatment and care of sick newborns has not solved all problems. Appropriate neonatal care may be more important for the development of mother-infant interaction and attachment than earlier believed. This may be especially true for socially and emotionally vulnerable families. Overload of the newborns by unnatural, evolutionary unforeseen stimuli such as apparatuses, many care-givers, painful procedures *etc.* may influence the developing neuronal networks and neuroendocrine responses and have long-term consequences.

A better understanding of newborn's needs and capacity may be obtained by observations of newborns in biologically meaningful situations, *e.g.* when they try to locate the mother's nipple for sucking after birth. Our findings suggest that when a newborn baby spontaneously crawls towards the breast it is using the same congenital reflexes as the physician examines during the neurological examination of the newborn. Such, genetically determined behaviors have in mammals a survival value: the animal with decreased or absent searching, sucking and crawling abilities will often die. The human newborn, incapacitated because of illness, prematurity or drugs given to the mother for pain relief during labor, survives because the mother can help the baby to the breast or because of tube feeding, respiratory support *etc.*, but could be disabled. It has been established that maternal administration of pethidin within 5 hrs before delivery (Nissen *et al.* 1997), infant gastric suction after birth (Widström *et al.* 1987) and a brief interruption of mother-infant contact (Righard and Alade 1990) disturb the sequence of innate behaviors mentioned above. We have found that bathing of the newborn shortly after birth may also be inappropriate: it removes from the skin attractive amniotic fluid that stimulates hand-mouth contact and finger sucking which is a part of the predictable pre-feeding behavior seen in unwashed newborns within the first postnatal hour. The latter might be a key behavior that contributes to the maturation and development of the child's digestive tract (Widström *et al.* 1987).

Innate spontaneous movements help the newborn to the breast. Prior to the research reported in this thesis we did not know which senses (visual, tactile, olfactory) the newborn uses to locate the nipple for sucking. Data from research in humans and other mammals indicate that newborns are able to recognize lactating females and, particulary, their own lactating mother by smell. We have shown that naturally occurring breast odors contribute to early nipple attachment and sucking. We have also found that a cotton pad carrying the mother's breast odor elicits crawling movements which bring the baby to the pad — thus, smell without support of any additional maternal cues is sufficient to attract the newborn. We have also seen that exposure to maternal breast odor during 31–90 min after birth causes increased crying. A reasonable hypothesis is that this odor made the baby's brain "expect" food. The unsatisfied baby began to cry. In contrast, newborns exposed to the smell of amniotic fluid cried less than unexposed controls. Futhermore, newborn babies preferred a breast coated with the mother's amniotic fluid rather than the naturally smelling breast. This suggests fetal olfactory learning and that the newborn can use this for early postnatal adaptive behavior. Familiarization with the odor of amniotic fluid *in utero* might also facilitate the early development of olfactory recognition of the mother. The olfactory recognition of the mother soon after birth may be the first specific tie between the mother and the baby. Because individual recognition is a necessary precursor of the development of specific social attachment, it could also contribute to infant-mother bonding.

The preference for amniotic fluid odor over breast odor waned during the first 2–5 days after birth and was replaced by a preference for breast odor. This change seemed to be due to postnatal experience and learning — the more time the baby spent with the mother, the earlier the baby preferred breast odor to amniotic fluid odor. Our data provide additional evidence that intensive olfactory postnatal learning takes place within the first days of life.

The importance of prenatal and early postnatal learning in the individual's development is discussed further by Hudson and Distel (1999). They report on the growing evidence from animal studies as well as ongoing human studies that early experience may have a role in shaping later chemosensory preferences and that fetal life and early infancy is a sensitive period for food-associated chemosensory learning.

Previous research used "artificial" testing situations such as head-turning to odorized pads. Our results were obtained during biologically meaningful experimental conditions and give evidence that naturally occurring odors play a significant role during early postnatal adaptation. Animal studies suggest that there may also be long-term behavioral effects.

Although there is still very little information about human neonatal behavior and olfaction, it is obvious that naturally occurring odors play a significant role in perinatal adaptation. Fetal olfactory experience and learning help the perinate to obtain information about maternal diet and food habits. This could be the first step in prenatal-postnatal olfactory continuity, ensuring the recognition of mother, establishment of successful sucking and effective breastfeeding, as well as subsequent social development. The innate capacities facilitate and direct early learning in order to allow newborn infants to understand their newly encountered world.

6. CONCLUSIONS

Naturally occurring odors have a significant influence on neonatal behavior.

Newborn babies crawl spontaneously to the breast and begin to suckle within an hour of birth. This behavior remains constant during the following days but the pace is accelerated when the babies are aroused by an expected breastfeeding. The genetically determined motor actions, which bring the babies to the breast and prepare them for sucking, are based on congenital primitive reflexes.

Naturally occurring maternal odors seem to have a role in guiding the newborn to the nipple and contribute to early nipple attachment and sucking.

Maternal breast odor collected on a pad and presented to the baby placed prone on a table, elicits the complete sequence of pre-feeding behaviors inclusive crawling towards the odor source. This suggests that odor stimuli do not need support from other sensory modalities (such as vision, sense of skin texture or temperature) when newborns spontaneously approach the breast and begin to suckle.

The human newborns like some other mammals, are attracted to the odor of amniotic fluid shortly after birth. Amniotic fluid odor even overrides the natural breast odor as an attractant. This postnatal response to amniotic fluid odor is evidence of prenatal learning.

Newborn babies, when placed on the mother's abdomen, develop a stereotype pre-feeding behavior, where bringing the hand to the mouth is an essential part. This behavior disappears when newborns are washed before they are put to the breast.

Odor preferences change during the first postnatal days. Thus, while amniotic fluid odor was preferred to maternal breast odor shortly after birth, at 2–4 postnatal days some babies still preferred amniotic fluid smell, but others breast smell. Those infants who preferred breast smell had spent longer time with their mothers and had had more breastfeeding opportunities. This suggests that the responsiveness to breast odors was enhanced by postnatal experience, *i.e.* postnatal olfactory learning. By the end of the first postnatal week all babies preferred breast odor to amniotic fluid odor.

Naturally occurring odors influence crying behavior in newborns separated from their mothers during the first 90 minutes after birth. Exposure to AF soothed crying babies. Exposure to maternal breast odor during 31-90 postnatal minutes, the time corresponding to naturally occurring breastfeed, caused an increased crying. We hypothesize that at this time breast odor made the brain "expect" sucking and milk delivery (the brain is programmed for suckling and milk when stimulated by breast odor). When this failed the baby became frustrated.

Olfaction has long been neglected as an important factor in the postnatal adaptation of the newborn. From a practical point of view one should probably avoid washing the breast before sucking. Since washing the baby, which eliminates amniotic fluid from the skin, disturbes the innate pre-feeding behavior, it might be prudent to avoid bathing the newborn before the first feed.

REFERENCES

Als H. Toward a synactive theory of development: Promise for the assessment and support of infant individuality. Infant Mental Health Journal 1982; 3: 229–243.

Altbacker V, Hudson R, Bilko A. Rabbit mother's diet influences pups' later food choice. Ethology 1995; 99: 107–116.

Amemiya F, Vos JE, Prechtl HFR. Effects of prone and supine position on heart rate, respiratory rate and motor activity in fullterm newborn infants. Brain Development 1991; 13: 148–154.

Antoshechkin AG, Golovkin AB, Maximova LA, Bakharev VA. Screening of amniotic fluid metabolites by gas chromatography — mass spectrometry. Journal of Chromatography 1989; 489: 353–358.

Axel R. The molecular logic of smell. Scientific American 1995; Oct: 130–137.

Balogh RD, Porter RH. Olfactory preferences resulting from mere exposure in human neonates. Infant Behavior and Development 1986; 9: 395–401.

Beauchamp GK, Katahira K, Yamazaki K, Mennella JA, Bard J, Boyse EA. Evidence suggesting that the odortypes of pregnant women are a compound of maternal and fetal odortypes. Proceedings of the National Academy of Sciences, USA 1995; 92: 2617–2621.

Beauchamp GK, Yamazaki K, Currant M, Bard J, Boyse EA. Fetal H-2 odortypes are evident in the urine of pregnant female mice. Immunogenetics 1994; 39: 109–113.

Berghard A, Buck LB. Sensory transduction in vomeronasal neurons: evidence for G alpha o, G alpha i2, and adenylyl cyclase II as major components of a pheromone signaling cascade. Journal of Neuroscience 1996; 16: 909–918.

Blass EM, Teicher MH, Cramer CP, Bruno JP, Hall WG. Olfactory, thermal, and tactile controls of suckling in preauditory and previsual rats. Journal of Comparative and Physiological Psychology 1977; 91: 1248–1260.

Blass EM, Teicher MH. Suckling. Science 1980; 210: 15–22.

Bossy J. Development of olfactory and related structures in stages human embryos. Anatomy and Embryology 1980; 161: 225–236.

Bowlby J. Attachment and loss: Vol. 1 Attachment. New York: Basic, 1969.

Brackbill Y, Douthitt TC, West H. Psychophysiological effects in the neonate of prone vs supine placement. Journal of Pediatrics 1973; 82: 82–83.

Bradley RM, Mistretta CM. Fetal sensory receptors. Physiological Reviews 1975; 55: 352–382.

Brennan P, Kaba H, Keverne EB. Olfactory recognition: a simple memory system. Science 1990; 250: 1223–1226.

Bruno JP, Teicher MH, Blass EM. Sensory determinants of suckling behavior in weanling rats. Journal of Comparative and Physiological Psychology 1980; 94: 115–127.

Budavari S (ed). The Merck Index 1989; Rahway, NJ: Merck & Co.

Burton JL, Shuster S, Cartlidge M, Libman LJ, Martell U. Lactation, sebum excretion and melanocyte-stimulating hormone. Nature 1973; 243: 349–350.

Butterworth G, Hopkins B. Hand-mouth coordination in the new-born baby. British Journal of Developmental Psychology 1988; 6: 303–314.

Cernoch JM, Porter RH. Recognition of maternal axillary odors by infants. Child Development 1985; 56: 1593–1598.
Christ JE and Meiniger MG. Ultrasound study of the nose and upper lip before birth. Annals of Plastic Surgery 1983; 11: 308–312.
Christensson K, Cabrera T, Christensson E, Uvnäs-Moberg K, Winberg J. Separation distress call in the human neonate in the absence of maternal body contact. Acta Paediatrica 1995; 84: 468–473.
Chuah MI, Zheng DR. Olfactory marker protein is present in olfactory receptor cells of human fetuses. Neuroscience 1987; 23: 363–370.
Chuah MI, Farbman AI. Developmental anatomy of the olfactory system. In: Doty RL (ed). Handbook of Olfaction and Gustation. New York: Marcel Dekker, 1995: 147–70.
Coude M, Chadefaux B, Rabier D, Kamoun P. Early amniocentesis and amniotic fluid organic acid levels in the prenatal diagnosis of organic acidemias. Clinica Chimica Acta 1990; 187: 329–332.
Davis LB, Porter RH. Persistent effects of early odor exposure in human neonates. Chemical senses 1991; 16: 169–174.
De Chateau P, Wiberg B. Long-term effect on mother-infant behaviour of extra contact during the first hour post partum. III. Follow-up at one year. Scandinavian Journal of Social Medicine 1984; 12: 91–103.
De Vries JIP, Visser GHA, Prechtl HFR. Fetal motility in the first half of pregnancy. In: Prechtl HFR (ed), Continuity of neural functions from prenatal to postnatal life. Spastics International Medical Publications 1984: 46–64.
DeCasper AJ, Fifer WP. Of human bonding: Newborns prefer their mother's voices. Science 1980; 208: 1174–1176.
DeCasper AJ, Sigafoos AD. The intrauterine heartbeat: a potent reinforcer for neonates. Infant Behavior and Development 1983; 6: 19–25.
DeCasper AJ, Spence MJ. Prenatal maternal speech influences newborns' perception of speech sounds. Infant Behavior and Development 1986; 9: 133–150.
Desmond MM, Franklin RR, Vallbona C, Hill RM, Plumb R, Arnold H, Watts J. The clinical behavior of the newly born. Journal of Pediatrics 1963; 62: 307–325.
Doty RL. Mammalian pheromones. In: Greenberg G, Haraway MH (eds). Comparative Psychology: A Handbook. New York: Garland, 1998: 313–320.
Duenholter JH, Pritchard JA. Fetal respiration: Quantitative measurements of amniotic fluid inspired near term by human and rhesus fetuses. American Journal of Obstetrics and Gynecology 1976; 125: 306–309.
Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. Cell 1995; 83: 195–206.
Edelstone DI, Merick RE, Caritis SN, Müller-Heubach E. Umbilical venous blood flow and its distribution before and during autonomic blockade in fetal lambs. American Journal of Obstetrics and Gynecology 1980; 138: 703–707.
Feijoo J. Le foetus Pierre et le loup ... ou une approche originale de l'audition prenatale humanine. In: Herbinet E, Busnel M-C (eds). L'aube des sens. Paris: Stock, 1981.
Feijoo J. Ut conscientia Noscatue. Cahier de Sophrologie 1975; 13: 14–20.
Feldman JF, Brody N. Non-elicited newborn behaviours in relation to state and prandial condition. Merrill-Palmer Quarterly 1978; 24: 79–84.

Fifer WP, Moon C. Psychobiology of newborn auditory preferences. Seminars in Perinatology 1989; 13: 430–433.
Fifer WP, Moon CM. The role of mother's voice in the organisation of brain function in the newborn. Acta Paediatrica 1994; Suppl 397: 86–93.
Galef BG, Muskus PA. Caecotrophe may be one of a variety of odors that attract the pups to the mother. Journal of Comparative and Physiological Psychology 1979; 93: 695.
Goodlin RC, Lowe EW. Multiphasic fetal monitoring. A preliminary evaluation. American Journal of Obstetrics and Gynecology 1974; 119: 341–357.
Goren CC, Sarty M, Wu PYK. Visual following and pattern discrimination of face-like stimuli by newborn infants. Pediatrics 1975; 56: 544–549.
Graham H. Eternal Eve, the history of gynecology and obstetrics. New York: Doubleday, 1951.
Granier-Deferre C, Lecanuet JP, Cohen H, Busnel MC. Feasibility of prenatal hearing test. Acta Otolaryngologica (Stockholm) 1985, Suppl 421: 93–101.
Hallak M, Cotton DB. Transfer of maternally administered magnesium sulfate into the fetal compartment of the rat: assessment of amniotic fluid, blood, and brain concentrations. American Journal of Obstetrics and Gynecology 1993; 169 (2Pt1): 427–431.
Hauser GJ, Chitayat D, Berns L, Braver D, Muhlbauer B. Peculiar odours in newborns and maternal prenatal ingestion of spicy food. European Journal of Pediatrics 1985; 144: 403.
Hepper PG, Leader LR. Fetal habituation. Fetal Medicine Review 1996; 8: 109–123.
Hepper PG, Shahidullah S. Development of fetal hearing. Archives of Disease in Childhood 1994; 71: F81–F87.
Hepper PG. Adaptive fetal learning: Prenatal exposure to garlic affects postnatal preferences. Animal Behavior 1988; 36: 935–936.
Hepper PG. An examination of fetal learning before and after birth. Irish Journal of Psychology 1991; 12: 95–107.
Hepper PG. Fetal memory: Does it exist? What does it do? Acta Paediatrica 1996; Suppl 416: 16–20.
Hepper PG. Fetal psychology: an embryonic science. In: Nijhuis JG (ed). Fetal behaviour: Developmental and perinatal aspects. Oxford: Oxford University Press, 1992: 129–156.
Hepper PG. Human fetal "olfactory" learning. International Journal of Prenatal and Perinatal Psychology and Medicine 1995; 7: 147–151.
Hepper PG. The amniotic fluid: an important priming role in kin recognition. Animal Behavior 1987; 35: 1343–1346.
Herrada G, Dulac C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell 1997; 90: 763–773.
Hofer MA, Fisher A, Shair H. Effects of intraorbital nerve section on survival, growth, and suckling behaviors of developing rats. Journal of Comparative and Physiological Psychology 1981; 95: 23–33.
Hudson R, Distel H. Nipple location by newborn rabbits: behavioural evidence for pheromonal guidance. Behavior 1983; 85: 260–275.

Hudson R, Distel H. The flavor of life: perinatal development of odor and taste preferences. Schweizerische Medizinische Wochenschrift 1999; 129: 176–181.
Humphrey T. Functions of the nervous system during prenatal life. In: Stave U (ed). Perinatal Physiology. New York: Plenum, 1978: 651–683.
Humphrey T. The development of the olfactory and the accessory olfactory formations in human embryos and fetuses. Journal of Comparative Neurology 1940; 73: 431–468.
Keverne EB. Olfactory learning. Current Opinion in Neurobiology 1995; 5: 482–488.
Kjellmer I, Winberg J. The neurobiology of infant-parent interaction in the newborn: an introduction. Acta Paediatrica 1994; Suppl 397: 1–2.
Kodama N. Preference for amniotic fluid in newborn mice. Annual meeting of the International Society of Developmental Psychobiology 1990; Cambridge, England.
Koepke JE, Bigelow AE. Observations of newborn suckling behavior. Infant Behavior and Development 1997; 20: 93–98.
Koepke JE, Pribram KH. Effect of milk on the maintenance of sucking behavior in kittens from birth to six months. Journal of Comparative and Physiological Psychology 1971; 75: 363–377.
Lagercrantz H, Slotkin TA. The "stress of being born". Scientific American 1986; April: 92–102.
Lagercrantz H. Excitation of the sympathoadrenal system at birth. In: Amiel-Tisson C, Steward A (eds). One Brain for Life: The newborn infant. Paris: INSERM, 1994: 57–66.
Lagercrantz H. Stress, arousal, and gene activation at birth. News Physiol Sci 1996; 11: 214–218.
Leader LR, Baille P, Martin B, Molteno C, Wynchank S. Foetal responses to vibrotactile stimulation. A possible predictor of foetal and neonatal outcome. Australian and New Zealand Journal of Obstetrics and Gynecology 1984; 24: 251–256.
Leader LR, Baillie P, Martin B, Vermeulen E. The assessment and significance of habituation to a repeated stimulus by the human fetus. Early Human Development 1982; 7: 211–219.
Lee CWG, Yu JS, Turner BB, Murray KE. Trimethylaminuria: fishy odors in children. New England Journal of Medicine 1975; 295: 937–938.
Leon M, Moltz H. The development of the pheromonal bond in the albino rat. Physiology and Behavior 1972; 8: 683–686.
Logvinenko AV. Recording of fetal respiratory movements based on studying the shifting of the amniotic fluid in the trachea by the Doppler effect. Akusherstvo i Ginekologia 1990; 7: 38–41.
Louis-Sylvestre J, Le Magnen J. A fall in blood glucose level precedes meal-onset in free feeding rats. Neuroscience and Biobehavioral Reviews 1980; 4: 13–16.
Macfarlane AJ. Olfaction in the development of social preferences in the human neonate. In: Porter R, O'Connor M (eds). Parent-infant interaction (Ciba Found Symp 33). New York: Elsevier, 1975: 103–113.
Makin JW, Porter RH. Attractiveness of lactating females' breast odors to neonates. Child Development 1989; 60: 803–810.
Marchini G, Linden A. Cholecystokinin, a satiety signal in newborn infants? Journal of Developmental Physiology 1992; 17: 215–219.

Marchini G, Persson B, Berggren V, Hagenäs L. Hunger behaviour contributes to early nutritional homeostasis. Acta Paediatrica 1998; 87: 671–675.
Marchini G, Persson B, Uvnäs-Moberg K. Metabolic correlates of behaviour in the newborn infant. Physiology and Behavior 1993; 54: 1021–1023.
Marchini G, Stock S. Thirst and vasopressin secretion counteract dehydration in newborn infants. Journal of Pediatrics 1997; 130: 736–739.
Marlier L, Schaal B, Soussignan R (1998-a). Neonatal responsiveness to the odor of amniotic and lacteal fluids. Child Development 1998, 69: 611–623.
Marlier L, Schaal B, Soussignan R (1998-b). Bottle-fed neonates prefer an odor experienced in utero to an odor experienced postnatally in the feeding context. Developmental Psychobiology 1998; 33: 133–145.
Marlier L, Schaal B. Familiaritç et discriminstion olfactive chez le nouveau-nç: influence differçntielle du mode d'alimentation? Enfance 1997; 1: 47–61.
Mehler H, Jusczyk P, Lambertz G, Halsted N, Bertonicini J. A precursor of language acquisition in young infants. Cognition 1988; 29: 143–178.
Menkes JH, Hurst PL, Craig JM. A new syndrome: progressive familial infantile cerebral dysfunction associated with an unusual urinary substance. Pediatrics 1954; 14: 462–467.
Mennella JA, Beauchamp GK. Maternal diet alters the sensory qualities of human milk and the nursling's behavior. Pediatrics 1991; 88: 737–744.
Mennella JA, Johnson A, Beauchamp GK. Garlic ingestion by pregnant women alters the odor of amniotic fluid. Chemical Senses 1995; 20: 207–209.
Meza CV, Powell NJ, Covington C. The influence of olfactory intervention on non-nutritive sucking skills in a premature infant. The Occupational Therapy Journal of Research 1998; 18: 71–83.
Michelsson K, Christensson K, Rothgänger H and Winberg J. Crying in separated and non-separated newborns: Sound spectrographic analysis. Acta Paediatrica 1996; 85: 471–475.
Moon C, Cooper RP, Fifer WP. Two-day-olds prefer their native language. Infant Behavior and Development 1993; 16: 495–500.
Morrow-Tesch J, McGlone JJ. Sensory systems and nipple attachment behavior in neonatal pigs. Physiology and Behavior 1990; 47: 1–4.
Mucignat-Caretta C, Caretta A, Cavaggioni A. Acceleration of puberty in female mice by male urinary proteins. Journal of Physiology 1995; 486 (Pt2): 517–522.
Nakashima T, Kimmelman CP, Snow JB. Structure of human fetal and adult olfactory neuro-epithelium. Archives of Otolaryngology — Head and Neck Surgery 1984; 110: 641–646.
Neville MC. Lactogenesis in women: A cascade of events revealed by milk composition. In: Jensen RG (ed). Handbook of milk composition. San Diego, CA: Academic Press, 1995: 87–98.
Nichols TM, Hähnel R, Wilkinson SP. Organic acids in amniotic fluid. Clinica Chimica Acta 1978; 84: 11–17.
Nissen E, Widström A-M, Lilja G, Matthiesen AS, Uvnäs-Moberg K, Jacobsson G, Boreus L. Effects of routinely given pethidine during labour on infants developing breast-feeding behaviour. Acta Paediatrica 1997; 86: 201–208.
Parfet KAR, Gonyou HW. Attraction of newborn piglets to auditory, visual, olfactory and tactile stimuli. Journal of Animal Sciences 1991; 69: 125–133.

Pedersen PA, Blass EM. Prenatal and postnatal determinants of the 1st suckling episode in albino rats. Developmental Psychobiology 1982; 15: 349–355.

Pedersen PA, Steward WB, Greer CA, Sheperd GM. Evidence for olfactory function in utero. Science 1983; 221: 478–480.

Pihet S, Mellier D, Bullinger A, Schaal B. Rëponses comportementales aux odeurs chez le nouveau-në preëmaturë: ëtude prëliminaire (Behavioral responses to odours in the premature infant: First data). Enfance 1997; 1: 33–46.

Plagge JC. American Journal of Physiology 1935; 212: 1288.

Porter RH, Makin JW, Davis LB, Christensen KM. An assessment of the salient olfactory environment of formula-fed infants. Physiology and Behavior 1991; 50: 907–911.

Porter RH, Makin JW, Davis LB, Christensen KM. Breast-fed infants respond to olfactory cues from their own mother and unfamiliar lactating females. Infant Behavior and Development 1992; 15: 85–93.

Porter RH, Sentell SW, Makin JW. Effects of intranasal $ZnSO_4$ irrigation are mitigated by the presence of untreated littermates. Physiology and Behavior 1987; 40: 97–102.

Porter RH, Winberg J. Unique salience of maternal breast odors for newborn infants. Neuroscience and Biobehavioral Reviews 1999; 23: 439–449.

Porter RH. Human reproduction and the mother-infant relationship: The role of odors. In: Getchell DV, Doty RL, Bartoshuk LM and Snow JB, Jr (eds). Smell and Taste in Health and Disease. New York: Raven Press, 1994: 429 –442.

Porter RH. Mutual mother-infant recognition in humans. In: Hepper PG (ed). Kin Recognition. Cambridge: Cambridge University Press 1991, 413–432.

Potts WK, Wakeland EK. Evolution of mhc genetic diversity: a tale of incest, pestilence and sexual preference. Trends in Genetics 1993; 9: 408–412.

Pyatkina GA. Development of the olfactory epithelium in man. Zeitschrift für Mikroskopie und Anatomische Forschung 1982; 96: 361–372.

Rasmussen LEL, Perris TE, Gunawardena R. Isolation of potential musth-alerting signals from the temporal glands secretions of male Asian elephants (*Elephas maximus*): a new method. Chemical senses 1994; 19: 540.

Rau H. Frühe Kindheit. In: Oerter R, Montada L (eds). Entwicklungs-psychologie. Wien: Urban & Schwarzenberg, 1982: 67–93.

Righard L, Alade MO. Effect of delivery room routines on success of first breast-feed. Lancet 1990; 336: 1105–1107.

Righard L, Alade MO. Sucking technique and its effect on success of breastfeeding. Birth 1992; 19: 185–189.

Robinson SR, Smotherman WP. Fetal learning: implications for the development of kin recognition. In: Hepper PG (ed). Kin Recognition. Cambridge: Cambridge University Press, 1991: 308–334.

Salk L. Mother's heart beat as an imprinting stimulus. Transactions of the Academy of Sciences of New York 1962; 24: 753–763.

Salk L. The effects of the normal heart beat sound on the behavior of the new-born infant: implications for mental health. World Mental Health 1960; 12: 168–175.

Sarnat HB. Olfactory reflexes in the newborn infant. Journal of Paediatrics 1978; 92: 624–626.

Scalia F, Winans SS. The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. Journal of Comparative Neurology 1975; 161: 31–56.

Schaal B, Marlier L. Maternal and paternal perception of individual odor signatures in human amniotic fluid — potential role in early bonding? Biology of the Neonate 1998; 74: 266–273.
Schaal B, Marlier L, Soussignan R (1995-c). Responsiveness to the odour of amniotic fluid in the human neonate. Biology of the Neonate 1995; 6–7: 397–406.
Schaal B, Marlier L, Soussignan R. Olfactory function in the human fetus: evidence from selective neonatal responsiveness to the odor of amniotic fluid. Behavioral Neuroscience 1998; 112: 1438–1449.
Schaal B, Montagner H, Hertling E, Bolzoni D, Moyse A, Quichon R. Les stimulations olfactives dans les relations entre l'enfant et la mere. Reproduction, Nutrition et Developpement 1980; 20: 843–858.
Schaal B, Orgeur P, Arnould C (1995-b). Olfactory preferences in newborn lambs: Possible influence of prenatal experience. Behaviour 1995; 132: 351–365.
Schaal B, Orgeur P, Rognon C (1995-a). Odor sensing in the human fetus: Anatomical, functional and chemo-ecological bases. In: Lecanuet JP, Krasnegor NA, Fifer WA, Smotherman WP (eds). Prenatal development: A psychobiological perspective. Hillsdale, NJ: Erlbaum, 1995: 205–237.
Schaal B, Orgeur P. Olfaction in utero: can the rodent model be generalised? Quarterly Journal of Experimental Psychology 1992; 44B: 245–278.
Schaal B. La competence olfactive du nouveau-ne humain: aspects fonctionnels. Psychologie Medicale 1984; 16: 305–314.
Schleidt M, Genzel C. The significance of mother's perfume for infants in the first weeks of their life. Ethology and Sociobiology 1990; 11: 145–154.
Seeds AE. Current concepts of amniotic fluid dynamics. American Journal of Gynecology and Obstetrics 1980; 138: 575–586.
Sehnal JP, Palmeri A. High-risk infants. In: Pratt PN, Allen AS (eds). Occupational therapy for children. St. Louis: C.V. Mosby, 1989.
Semke E, Distel H, Hudson R. Specific enhancement of olfactory receptor sensitivity associated with foetal learning of food odours in the rabbit. Naturwissenschaften 1995; 82: 148–149.
Singh PJ, Hofer MA. Olfactory cues in nipple orientation and attachment in rat pups. Neuroscience abstracts 1976; 2: 163.
Smith TD, Siegel MI, Mooney MP, Burdi AR, Burrows AM, Todhunter JS. Prenatal growth of the human vomeronasal organ. Anatomical Record 1997; 248: 447–455.
Smotherman WP, Robinson SR. Psychobiology of fetal experience in the rat. In: Krasnegor NA, Blass EM,. Hofer MA, Smotherman WP (eds). Perinatal Development: A Psychobiological Perspective. Orlando: Academic Press, 1994: 39–60.
Smotherman WP. (1982-a). In utero chemosensory experience alters taste preferences and corticosterone responsiveness. Behavioral and Neural Biology 1982; 36: 61–68.
Smotherman WP. (1982-b). Odor aversion learning by the rat fetus. Physiology and Behavior 1982; 29: 769–771.
Stafford M, Horning MG, Zlatkis A. Profiles of volatile metabolites in body fluids. Journal of Chromatography 1976; 126: 495–502.
Steiner JE. Facial expressions of the neonate infant indicating the hedonics of food-related chemical stimuli. In: Weiffenbach, JM (ed). Taste and Development. The Genes and Sweet Preference. DHEW Publication, 1977.

Stern K, McClintock M. Regulation of ovulation by human pheromones. Nature 1998; 392: 177–179.
Stoddard DM. The scented ape. The biology and culture of human odour. Cambridge: Cambridge University Press, 1991.
Stoloff ML, Kenny JT, Blass EM, Hall WG. The role of experience in suckling maintenance in albino rats. Journal of Comparative and Physiological Psychology 1980; 94: 847–856.
Sullivan RM, Zyzak DR, Skierkowski P, Wilson DA. The role of olfactory bulb norepinephrine in early olfactory learning. Developmental Brain Research 1992; 70: 279–282.
Sullivan RM, Toubas B. Clinical usefulness of maternal odor in newborns: soothing and feeding preparatory responses. Biology of the Neonate 1998; 74: 402–408.
Teicher MH, Blass EM. Suckling in newborn rats: Eliminated by nipple lavage, reinstated by pup saliva. Science 1976; 193: 422–425.
Teicher MH, Blass EM. First suckling response of the newborn albino rat: The role of olfaction and amniotic fluid. Science 1977; 198: 635–636.
Thompson RF, Spencer WA. Habituation: A model for the study of neuronal substrates of behavior. Psychological Review 1966; 73: 16–43.
Tirindelli R, Mucignat-Caretta C, Ryba NJP. Molecular aspects of pheromonal communication via the vomeronasal organ of mammals. Trends in Neuroscienses 1998; 21: 482–486.
Uvnäs-Moberg K, Marchini G, Winberg J. Plasma cholecystokinin concentrations after breast feeding in healthy 4 day old infants. Archives of Disease in Childhood 1993; 68 (1 Spec No): 46–48.
Uvnäs-Moberg K, Winberg J. Role for sensory stimulation in energy economy of mother and infant with particular regard to the gastrointestinal endocrine system. In: Lebenthal E (ed). Textbook of gastroenterology and nutrition in infancy. New York: Raven Press, 1989: 53–62.
Walton GE, Armstrong ES, Bower TGR. Faces as forms in the world of the newborn. Infant Behavior and Development 1997; 20: 537–543.
Wedekind C, Seebeck T, Bettens F, Paepke AJ. MHC-dependent mate preferences in humans. Proceedings of the Royal Society of London, Series B 1995; 260: 245–249.
Weller A. Communication through body odour. Nature 1998; 392: 126–127.
Widström AM, Ransjö-Arvidson AB, Christensson K, Matthiesen A-S, Winberg J, Uvnäs-Moberg K. Gastric suction in healthy newborn infants. Acta Paediatrica Scandinavica 1987; 76: 566–572.
Widström A-M, Tingström-Paulsson J. The position of the tongue during rooting reflexes elicited in newborn infants before the first suckle. Acta Paediatrica Scandinavica 1993; 82: 281–283.
Winans SS, Scalia F. Amygdaloid nucleus: new afferent input from the vomeronasal organ. Science 1970; 170: 325–344.
Vince MA, Ward TM. The responsiveness of newly born Clun forest lambs to odour sources in the ewe. Behaviour 1984; 89: 117–127.
Woolridge MW. The anatomy of infant sucking. Midwifery 1986; 2: 164–171.
Wysocki CJ, Lepri JJ. Consequences of removing the vomeronasal organ. Journal of Steroid Biochemistry and Molecular Biology 1991; 39: 661–669.

SUMMARY IN ESTONIAN

VASTSÜNDINU KÄITUMINE LOOMULIKE LÕHNADE KESKKONNAS

Paljude uuringutega on tõestatud, et imetajate partnerivalik, seksuaalkäitumine, järglaste äratundmine ja nende side vanemate ning maailmaga toimub lõhnade ja feromoonide kaasabil. Primaatide klassi kuuluval imetajal inimesel on leitud vähemalt üks feromoon, samuti hiirtega analoogne võime valida kehalõhnade järgi sellise geneetilise koodiga partner, kellega saaks parima immuunkompetentsusega järglasi. Meie genoomist 1% kontrollib haistmisretseptoreid. Haistmisaistingutel on ilmselt suur mõju inimese füsioloogiale ja emotsioonidele, mis võimaldub otseühenduse tõttu aju kognitiivsete keskustega.

Järglaste saamine, nende eest hoolitsemine ning vastsündinute käitumine ellujäämise nimel kuuluvad looduse peaprogrammide hulka. Nimetatud funktsioone reguleerivad enamasti vanemad ajuosad ja nad toimivad paljudel imetajatel sarnaselt, mis annab võimaluse uurida inimese neurobioloogilisi funktsioone teiste imetajate käitumisanaloogiate alusel.

Lootel on kindlaks tehtud võimeid, mis valmistavad teda ette kohanemiseks üsavälise maailmaga ning õpetavad tundma oma ema ja emakeelt, näiteks õppimine kuulmise, maitsmise ja haistmise kaudu ning vastav mälu. Loote haistmisvõimet ja sünnieelset õppimist, mille üks tunnus on vastsündinu reaktsioon looteveele, oli enne meie uuringuid näidatud rohketes loomkatsetes.

Enne meie töö algust uuriti vastsündinu lõhnaeelistusi sel moel, et tema pea külgedele riputati erisuguse lõhnaga marlilapid ja vaadati, kummale poole laps pea pöörab. Nii on tuvastatud, et lakteeriva naise rinna lõhn sisaldab vastsündinu jaoks kaht tüüpi signaale: 1) unikaalseid, ainult ühele naisele omaseid lõhnu, et aidata vastsündinul ära tunda oma ema; 2) üldisi lõhnu, mis on vastsündinule atraktiivsed, sõltumata haistmis- ja toitmiskogemusest. Seega on haistmine väga oluline toitmis-imemisahela ning ema-vastsündinu sideme kinnitaja.

Vastsündinu võime spontaanselt ema rinnale roomata taasavastasid Rootsi ämmaemandad kümmekond aastat tagasi, kui nad panid lapse kohe pärast sündi ema kõhule. Pärast puhke-lõõgastuspausi muutus vastsündinu aktiivseks: otsis suuga, tegi imemisliigutusi, keeras pead küljelt küljele, pani kätt suhu ja imes sõrme, kuni u. 55 minuti vanuselt ronis ema rinnanibuni ja hakkas imema. Sellist käitumist häirib ema-lapse nahakontakti katkestamine mõneks minutiks, vastsündinu maosisu aspireerimine ja petidiini manustamine sünnitajale.

Et vastsündinu eest paremini hoolitseda, on vaja teada, mida ta tegelikult vajab. Tema spontaanse toitmiseelse käitumise uurimine võib seega anda olulist informatsiooni.

Eesmärgid

Käesoleva uurimuse eesmärgiks oli selgitada loomulike lõhnade (ema rinna ja lootevee lõhn) võimalikku mõju vastsündinu käitumisele ning uurida vastsündinu toitmiseelse käitumise iseärasusi esimestel elupäevadel.

Metoodika

Kokku uuriti 186 ajalist tervet vastsündinut vanuses 0–10 päeva. Enamikul uuringutel oli ühesugune metoodika: vastsündinu asetati lamava ema kõhule kummuliasendisse. Enne seda ema ühte rinda "töödeldi" — kas peitsiti looteveega või pesti, et eemaldada rinna loomulik lõhn. Teine rind säilitas oma loomuliku lõhna. Jälgiti vastsündinu käitumise iseärasusi: pea tõstmine ja pööramine, käe-suu kontakt ja selle kestus, aktiivse otsimise algus, lapse suu esimene kontakt ema rinnaareooliga, rinnanibu imemise algus. Lõhnaeelistuse hindamiseks märgiti, kummale rinnale vastsündinu imemiseks liikus.

Selgitamaks, kas ema rinnalõhn võib ilma ema teiste stiimuliteta vallandada vastsündinu toitmiseelset käitumist ja liikumist lõhnaallika suunas, paigutati laps ema kõhu asemel soojendusvoodile. Ühe testi ajal pandi tema ninast 20 cm kaugusele lõhnatu vatipadi, teisel aga ema rinnal hoitud padjake. Lisaks käitumise jälgimisele mõõdeti testi vältel lapse ninaotsa asukohta, võrreldes algasendiga, et hinnata, kas laps liikus lõhnaallika suunas või ei.

Loomulike lõhnade mõju vastsündinu üksindusnutule uuriti järgmiselt: pärast sündi soojenduslambi alla asetatud vastsündinu nina juurde paigutati kas sünnituse ajal ema rinnal hoitud või lapse looteveega immutatud riie; kontrollgrupi lastele ei pandud midagi. Lapse häälitsused 31.–90. eluminutil salvestati helilindile, millelt hiljem mõõdeti nutu kestus.

Peamised tulemused ja järeldused

Enne käesoleva uurimuse algust oli vastsündinu spontaanset käitumist ema rinnal uuritud vaid vahetult sünnijärgsel perioodil. Meie tuvastasime, et spontaanne toitmiseelse käitumise programm töötab vähemalt 10. elupäevani. Vastsündinu käitumismudel on ka hiljem samasugune kui esimesel sünnijärgsel tunnil: laps otsib suuga, pöörab pead küljelt küljele, tõukab jalgadega ning lükkab end rinnale. Selline käitumine meenutab mitmete teiste imetajate vastsündinute tegevust enne rinnanibuni jõudmist. Liigutustest domineerivad need, mida uuritakse lapse läbivaatusel kaasasündinud reflekside nime all: otsimine, imemine, kaitserefleks (pea tõstmine ja pööramine küljele) ning roomamine. Nende reflekside puudumine viitab neuroloogilisele kahjustusele. Evolutsiooniliselt võis see tähendada, et vastsündinu ei suuda liikuda toiduallikani, ei saa süüa ning hukkub. Nii juhtub sageli haigete loomadega.

Vastsündinu spontaanne käitumine kohe pärast sündi ja hilisemas vanuses erineb aktiivsuse ja tempo poolest. Pärast sündi muutuvad lapsed aktiivsemaks ning hakkavad tegema otsimisliigutusi umbes pool tundi pärast emale asetamist ja jõuavad rinnale esimese elutunni lõpuosas. Alates teisest elupäevast jõuavad lapsed rinnale paari minuti jooksul pärast emale asetamist, kui nad on vähemalt kaks tundi pärast eelmist toidukorda ise ärganud ja loodavad süüa saada. Toitmiseelse käitumise aktiivsus ja tempo võivad sõltuda vastsündinu füsioloogilistest näitajatest (näiteks nälg või veresuhkru tase) ning väljendada tema hetkevajadusi.

Seega saab vastsündinut ema kõhul jälgida bioloogiliselt tähtsas kontekstis: ta saab käituda nii nagu hetkel vajalik ja realiseerida oma kaasasündinud programmi, mille eesmärgiks on imemiseks valmistumine ja rinnale jõudmine. Valikuga kahe erinevalt lõhnava rinna vahel näitab ta oma lõhnaeelistust, mis on hea meetod loomulike lõhnade olulisuse uurimiseks.

Esimesest eksperimendist selgus, et enamik lapsi liikus loomulikult lõhnavale, mitte pestud rinnale, mis tähendab, et vastsündinu orienteerub esimesel elutunnil rinnanibu lokaliseerimisel selle naturaalse lõhna järgi. Et ka 2–5-päevaste laste analoogilise uurimise tulemus oli samasugune, saab järeldada, et ema naturaalne rinnalõhn aitab vastsündinul orienteeruda spontaanse toitmiseelse käitumise ajal rinnanibu leidmisel, samuti on vajalik nibu õigel haaramisel ja imemisel.

Leidsime, et ema rinnalõhn tema enda juuresolekuta on söömiseks ärganud vastsündinule piisav stiimul, et vallandada lapse spontaanset liikumist ema rinnapadjakese suunas. Kontrollpadjakese juuresolekul laps pärast kõhuli panekut esialgu küll pöörab pead ja otsib, aga ei liigu paigalt.

Uurisime laste reaktsioone ka teisele loomuliku lõhna allikale — looteveele. Vastsündinud eelistasid pärast sündi liikuda looteveega kaetud rinnale, mis näitab, et lootevee lõhn on vastsündinule atraktiivne ja ületab ema naturaalse rinnalõhna või potentseerib seda. Lootevee eelistamine vastsündinu poolt kohe pärast sündi peegeldab sünnieelset kogemust ja haistmisõppimist, samuti inimvastsündinu võimet kasutada sünnieelset haistmismälu ema rinnal orienteerumisel ja rinnanibu lokaliseerimiseks. See sarnaneb loomvastsündinute võimega, mis rotipoegadel otsustab ellujäämise.

Lootevee atraktiivsuse uurimisel pesime vastsündinut, et tema kehal olev lootevesi ei mõjutaks valikut. Ilmnes, et pestud lapsed imesid väga harva oma kätt või sõrme, erinevalt pesemata vastsündinutest. Lootevesi käenahal võib olla stiimuliks, et vastsündinu imeks oma kätt sünnijärgse käitumisprogrammi ajal, mis võib olla vajalik seedimise ettevalmistamiseks enne ternespiima imemist.

Vanuses 2–5 elupäeva ei ilmnenud tõepäraseid erinevusi looteveega kaetud või loomulikult lõhnava rinna eelistustes. Vastsündinuid, kes eelistasid looteveega kaetud rinda, testisime umbes neli päeva hiljem täpselt samades katsetingimustes (looteveega kaeti ema seesama rind, mis esimesel korral, et vältida viga vasaku või parema poole eelistamise tõttu). Korduskatses muutsid kõik testitud lapsed oma lõhnaeelistust ning valisid imemiseks eelmise testiga võrreldes vastaspoolse, loomulikult lõhnava rinna. Seega muutuvad vastsündinu lõhnaeelistused esimesel elunädalal: algul domineeriv lootevee valik asendub 1. elunädala lõpuks loomulikult lõhnava rinna valikuga. Emade täidetud lapse toitmis- ja hooldamispäevikutest selgus, et loomuliku lõhnaga rinna eelistamine 2.–5. elupäeval oli positiivses korrelatsioonis vastsündinu vanuse ja testieelse kogemusega, s.t. ajaga, mille laps veetis ema ja tema rinnaga lähikontaktis. See viitab sünnijärgsele intensiivsele lõhnade õppimisele. Vastsündinu valikut mõjutab niisiis ema lõhnade suurenev ekspositsioon ja prenataalsete lõhnakomponentide puudumine või vähesus vastsündinu ümbruses.

Kokkupuude lootevee ning selle komponentidega looteeas võib hõlbustada vastsündinul ema äratundmist, indiviidi tundmine on aga tähtis eeldus sotsiaalsete suhete arenemiseks. Ema ja lapse sideme tugevnemisele pannakse alus väga varases sünnijärgses perioodis, mil ema äratundmine lõhna järgi võib olla esimeseks tähtsaks mõjuriks.

Vastsündinu üksindusnuttu esimestel elutundidel saab mõjutada loomulike lõhnadega: kui lapse lähedusse asetati tema enda looteveega immutatud riie, nutsid nad tõepäraselt vähem kui ema rinnalõhnale eksponeeritud või kontrollgrupi lapsed. Tulemus peegeldab sünnieelset õppimist ja sünnijärgset äratundmist ning rahunemist tuttava lõhna keskkonnas. Lapsed nutsid ema rinnalõhna läheduses kogu uuringu vältel rohkem kui lootevee- ja kontrollgrupp, ilmselt seoses ärritusega, mida rinnalõhna haistmine ilma imemisvõimaluseta neis esile kutsus. Käesoleva uuringu kontrollgrupi lapsed nutsid palju vähem kui sarnases, Madriidis tehtud katses (Christensson jt. 1996). Uuringutingimuste võrdlemisel ilmneb, et hooldamise eripäraga (lapse mähkimine ja soojendamine soojenduslambi all nagu meie katses) saab leevendada vastsündinu sünnijärgset üksindusstressi, kui tema eraldamine emast on möödapääsmatu.

Loote ja vastsündinu keskkonna loomulikel lõhnadel on perinataalses adaptatsioonis suur tähtsus. Loote haistmiskogemus ja õppimine haistmise teel annavad talle informatsiooni ema toitumisest ja harjumustest, mis võib olla oluliseks etapiks indiviidi toitumisharjumuste kujunemisel. Loote kogemus on esimeseks sammuks haistmise sünnieelses ja -järgses järjepidevuses. See kindlustab ema äratundmise, õige imemise ning rinnaga toitmise, samuti lapse adekvaatse sotsiaalse arengu. Kaasasündinud võimed hõlbustavad ja juhivad varast õppimist ning aitavad vastsündinul ümbritsevas maailmas kohaneda ja seda mõista.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to all those who have helped me during the course of my work.

Special thanks to:

- All infants and their mothers who participated in the study.
- Professor Jan Winberg, my main tutor. without his enthusiastic encouragement, support, inspiration, great patience, understanding and constructive criticism no thesis would have been produced.
- Associate Professor Anne Ormisson, my tutor, who introduced me into neonatology and research and shared her knowledge, for inspiration, encouragement, support and valuable discussions.
- Professor Richard H Porter, for inspiration, indispensable advice, sharing his time and knowledge in guiding me into the exciting world of olfaction and animal research, as well as the excellent linguistic help.
- Professor Tiina Talvik, for constructive criticism, advice and support.
- Professor Bengt Björksten, for support and introducing me into the world of "research is fun".
- Professor Marika Mikelsaar, for introducing me into the research and for teaching accuracy and consistency, staying optimistic at the same time.
- Kyllike Christensson, for interesting collaboration, valuable discussions, kind support and hospitality.
- Paediatric and nursing staff of the neonatal unit in Tartu University Women's Clinic, supervised by Dr. Galina Kudrjavtseva, for help and friendly atmosphere.
- Obstetricians and midwives of the Tartu University Women's Clinic, supervised by Dr. Viivi Kull, for their courteous help in observations and kind collaboration.
- Dr. Tiina Vinni and Mrs. Maie Piiroja, for kind technical assistance.
- Colleagues from Tartu University Children's Hospital for their support.
- My husband Virko and our daughters Kertu, Laura, Mari-Leen and Ave Liis for their constant support, understanding and patience with all my studies, papers and sometimes the lack of good mothering.
- My mother Liina for her constant support and self-denying help to keep house and garden and look after the children, my father Aatso and my brother Harras for support and understanding.

- My husband's parents, Helvi and Taavet, for their endless support and help, for being ideal loving grandparents for the children.

The study was supported by grants from the foundation "Frimurare Barnhuset", Karolinska Institute and Tartu University (ARLA 0475).

PUBLICATIONS

I

Varendi H., Porter R. H., Winberg J.
Does the newborn baby find the nipple by smell?
Lancet 1994; 344: 989–990.

Does the newborn baby find the nipple by smell?

H Varendi, R H Porter, J Winberg

We studied the involvement of naturally occurring odours in guiding the baby to the nipple. One breast of each participating mother was washed immediately after delivery. The newborn infant was placed prone between the breasts. Of 30 infants, 22 spontaneously selected the unwashed breast. The washing procedure had no effect on breast temperature. We concluded that the infants responded to olfactory differences between the washed and unwashed breasts.

Lancet 1994; **344:** 989–90

Healthy newborn infants placed between their mother's breasts locate the nipple without assistance and typically begin to suck within the first hour after birth.[1] Newborn rats, for example, fail to locate the nipple if their sense of smell is experimentally disrupted[2] or if the mother's ventrum is thoroughly washed.[3] Breastfed human infants responded differentially to a gauze pad that had been worn over their mother's nipple compared with an odorised pad from an unfamiliar woman, indicating that they recognise their mother's scent.[4,5] Breast odours from lactating women are generally attractive to infants, regardless of their mode of feeding.[6,7] At 2 weeks of age, babies that had been bottle-fed since birth spent more time turned toward the breast pad from an unfamiliar nursing mother than in the direction of a pad treated with their familiar formula.[8] Could chemical cues associated with the areola and nipple be implicated in initial (unassisted) nipple localisation and sucking?[1] To assess this hypothesis, nipple searching was observed after one of the mother's breasts had been thoroughly washed, thereby partly eliminating natural odour.

36 full-term babies born of mothers without analgesia and who had no postpartum complications were recruited. 6 were subsequently excluded because of disturbed nasal breathing (4) or failure to respond to the mother's breast during a test session (2). The final sample was 14 girls and 16 boys (gestational age 37–41 weeks; mean birthweight 3676 [SD 423] g; Apgar scores >7 at 1 and 5 min).

Immediately after birth, the baby was dried, laid skin-to-skin on the mother's abdomen and covered with a blanket. An assistant washed one of the mother's breasts for about 15 cm around the nipple with an odourless liquid soap: Mildtvål for 15 mothers and Lactacyd (Sanofi, pH 3·5) for the remaining 15. The washed breast was rinsed with lukewarm water and dried. The right breast was washed in 15 cases and the left breast in the others. Mothers were randomly assigned to the two soaps and to the right/left sides.

After the washed breast was dried, the observer (unaware of which breast had been cleaned) entered the room and moved the baby from the mother's abdomen to the "start" position—ie, nose in the midline of the mother's chest, eyes at the level of the nipples. Observation and recording of the baby's behaviour then began (5–13 min after birth; mean 9·9) and continued until the infant found the nipple and began to suck vigorously.

Before delivery, the mother was told that she and the observer would watch whether the infant could find the nipple without help. Mothers were instructed not to tell the observer which breast had been washed. Informed consent was obtained from all mothers, and fathers when present, and the study was approved by the local ethics committee.

To study any effect of the washing on breast temperature, an additional 6 mothers underwent the same washing within 12 hours after delivery. Surface temperatures were recorded at the nipple, border between the areola and white breast-skin, and midway between the areola and centre of the chest at 5–10, 15–20, 30–35, and 50–55 min.

25 infants spontaneously grasped a nipple and sucked (median latency 51 min after birth [range 22–100]). The other 5 required assistance in grasping the prolapsed nipple; the baby had mouth contact with one of the nipples and repeatedly attempted to grasp it before the observer assisted by placing the nipple in the infant's mouth (median latency 54 min after birth [22–85]). None of these 5 infants attempted to grasp the alternative nipple. 22 (12 boys, 10 girls) of the 30 infants selected the unwashed breast. The unwashed breast was selected by 10 infants in the Mildtvål and 12 in the Lactacyd soap groups. The table shows median times for other early behaviours.

The median temperature of washed breasts was slightly above that of unwashed breasts (maximum median difference was 0·5°C).

Within 1–2 h after birth, babies preferred their mother's unwashed breast, which is consistent with studies of non-human mammals showing that washing the mother's nipples/ventrum resulted in disturbed nipple finding and feeding. We excluded the possibility that our infants had responded to differences in the warmth of the two breasts.

Activity	Infants who chose:	
	Unwashed breast (n=22)	Washed breast (n=8)
Onset of active rooting	20 (11–41)	19 (10–30)
First hand-to-mouth movement	23 (10–60)	20 (7–29)
No of head turnings before choice of side	1 (0–6)	2 (0–6)
First mouth-areola contact	50 (19–99)	44 (22–72)
First successful nipple contact (in mouth)	51 (22–100)	45 (22–73)
No of sucking attempts before success	5 (1–21)	4 (1–7)
Sucking latency	54 (22–100)	48 (22–73)

Median (range) time (min) after birth.

Table: **Early postnatal behaviour in 30 newborn infants**

We explain the preference for the unwashed breast in that the infants were responding to differences in smell. Besides secreting milk and colostrum, the nipple/areola is dense in glands that might secrete attractive odours. The washing would have reduced or eliminated such cues, which is consistent with infants orienting preferentially towards a gauze pad contaminated with the breast odour of a lactating woman compared with an odourless control pad.[4,6] Naturally occurring maternal odours may have a role in guiding the neonate to the nipple, and thereby contribute to early nipple attachment and sucking. The causes of early failures of breastfeeding are often unknown.[9] Since breastfeeding is sensitive to even minor disturbances of the spontaneous interactions between mother and baby,[10] biological factors should be given attention. Unnecessary routine cleaning may interfere with the establishment of successful early breastfeeding by elimination of the infant's access to biologically relevant chemical signals.

This study was aided by grants from the Foundation "Frimurare Barnhuset" and from the Karolinska Institute.

References

1 Widström AM, Ransjo-Arvisson AB, Christensson K, et al. Gastric suction in healthy newborn infants. *Acta Paediatr* 1987; **76:** 566–72.
2 Teicher MH, Flaum LE, Williams M, Eckhert SJ, Lumia AR. Survival, growth and suckling behavior of neonatally bulbectomized rats. *Physiol Behav* 1978; **21:** 553–61.
3 Hofer MA, Shair H, Singh P. Evidence that maternal ventral skin substances promote suckling in infant rats. *Physiol Behav* 1976; **17:** 131–36.
4 Macfarlane A. Olfaction in the development of social preferences in the human neonate. In: Porter R, O'Connor M, eds. Parent-infant interaction (Ciba Foundation Symposium 33). New York: Elsevier, 1975: 103–13.
5 Schaal B, Montagner H, Hertling E, et al. Les stimulations olfactives dans les relations entre l'enfant et la mere. *Reprod Nutr Dev* 1980: **20:** 843–58.
6 Makin JW, Porter RH. Attractiveness of lactating females' breast odors to neonates. *Child Dev* 1989; **60:** 803–10.
7 Porter RH, Makin JW, Davis LB, Christensen KM. Breast-fed infants respond to olfactory cues from their own mother and unfamiliar lactating females. *Infant Behav Dev* 1992; **15:** 85–93.
8 Porter RH, Makin JW, Davis LB. Christensen KM. An assessment of the salient olfactory enironment of formula-fed infants. *Physiol Behav* 1991; **50:** 907–11.
9 Goldman AS, Garza C. Future research in human milk. *Pediatr Res* 1987; **22:** 493–96.
10 Righard L, Alade MO. Effect of delivery room routines on success of first breast-feed. *Lancet* 1990; **336:** 1105–07.

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II

Varendi H., Porter R. H., Winberg J.
Attractiveness of amniotic fluid odor: evidence of prenatal
olfactory learning?
Acta Paediatrica 1996; 85: 1223–1227.

Acta Pædiatr 85: 1223–7. 1996

Attractiveness of amniotic fluid odor: evidence of prenatal olfactory learning?

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Varendi H, Porter RH, Winberg J. Attractiveness of amniotic fluid odor: evidence of prenatal olfactory learning? Acta Pædiatr 1996;85:1223–7. Stockholm. ISSN 0803-5253

Human infants are responsive to maternal odors beginning shortly after birth. In several non-human mammals, the fetus is capable of olfactory learning and in some species neonates are attracted to the odor of amniotic fluid (AF). The present study examined the responses of newborn babies to AF in a biologically relevant context, i.e. during their initial attempt to locate the mother's nipple/areola. We observed newborns' spontaneous choice between a breast with the nipple/areola moistened with AF and an untreated breast; 23 of 30 infants chose the AF-treated breast. All babies had been washed before the observations, and only 12/30 sucked their hands/fingers prior to approaching the nipple/areola. In a previous study with unwashed newborns, the corresponding proportion was 27/30 ($p < 0.001$). We tentatively suggest that the observed attraction to AF odor may reflect fetal exposure to that substance (i.e. prenatal olfactory learning). Because of the salience of biological odors for neonates, products that eliminate or mask such cues should be avoided during the perinatal period.
□ *Amniotic fluid odor, newborn, olfaction, postnatal behavior, prenatal learning*

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Newborn humans can retain some memory trace of their prenatal experience, but such fetal learning has been demonstrated for only one sensory modality, viz. audition. Thus, neonates recognize acoustic stimuli to which they had been exposed *in utero* (1, 2). In several non-human mammalian species, the fetus is capable of olfactory learning; the newborn recognize the odor of substances that have been introduced into the amniotic fluid (AF) or ingested by their pregnant mother (3–5). Moreover, some newborn mammals are attracted to the natural odor of AF per se (6–8), and rat pups achieve initial contact with a nipple by orienting to AF that their mother had deposited on her ventrum (9, 10).

To date, there is no direct evidence of comparable olfactory learning by human fetuses, but histological and histochemical studies suggest that human fetal chemoreceptors are well developed by the last trimester (11). After approximately 6 months' gestation, AF begins to flow through the oral-nasal passages, thereby providing potential access to nasal receptors (12). Two-day-old infants orient preferentially to gauze pads bearing the odor of AF, which indicates that they may be familiar with that scent (13). We have assessed the response of newborn infants to the odor of AF in a biologically relevant context, i.e. when attempting to locate the nipple and suck for the first time. Neonates are attracted to naturally occurring maternal breast odors (14, 15) and such olfactory cues play a role in guiding the newborn to the nipple for the initial feeding bout within the first hour after birth (16, 17). We hypothesized that AF may enhance the attractiveness of odors emanating from the mother's breasts.

Patients and methods

An initial population of 32 full-term neonates born of healthy non-smoking mothers, with normal pregnancy and normal vaginal delivery without analgesia was recruited for this study. One infant was excluded 15 min after observation began because of disturbed nasal breathing. The final sample therefore consisted of 31 babies, 13 girls and 18 boys (gestational age 38–41 weeks; mean birthweight 3593 ± 336 g (range 2980–4410 g); Apgar scores > 7 at 1 and 5 min) (Table 1).

After birth of the baby a sterile gauze pad (~ 7 × 7 cm) was moistened with the mother's own AF collected in a receptacle ($n = 24$).

If the AF (postwater) in the receptacle contained blood as a result of rupture of the mother's perineum, it was not used because the blood might mask the AF odor. Instead, in these cases ($n = 7$), AF was gently wiped off the baby's body in an area where blood or vernix caseosa was not seen. We cannot exclude the possibility that the baby's own smell may also have been transferred to the pad. We attempted to achieve similar moisture of the pads in both methods. These pads were used in the experiment.

Table 1. Prefeeding behavior and sucking latency of infants choosing the amniotic fluid (AF) treated breast and those choosing the "natural odor"(Nat) breast.

		Choice of AF breast ($n = 23$)	Choice of Nat breast ($n = 7$)
Boys/girls*		13 / 10	4/3
birthweight, g	Median	3500	3568
	Range	2980–4410	3090–4150
Onset of observation,** minutes after birth	Median	12	15
	Range	7–23	6–21
Onset of active rooting, minutes after birth	Median	25	30
	Range	11–66	16–58
First hand-to-mouth contact, minutes after birth	Median	32 ($n = 8$)	29.5 ($n = 4$)
	Range	14–45	22–45
Number of head turnings before choosing the breast	Median	2	2
	Range	1–17	1–7
First mouth-areola contact, minutes after birth	Median	42	51
	Range	17–85	28–102
Sucking latency, minutes after birth	Median	46.5	52
	Range	17–88	38–103
	mean	49	61
	(±SD)	(20)	(26)
AF on left / right breast		11 L/12 R	4 L / 3 R
First head turn to left/right breast		9 L / 14 R	4 L / 3R
Final choice of left/right breast		11 L / 12 R	3 L / 4 R

*One baby is not included in the calculations since he showed no interest in finding the breast. Onset of active rooting at 44 min and first hand-mouth contact at 41 min. No mouth-areola contact.

**First mother infant body contact.

Immediately after birth the cord was clamped and cut, and the baby bathed in warm water (~37°C), with ~0.05% potassium permanganate. This routine is used in the delivery ward for desinfection purposes. The baby was dried and cleansed of vernix caseosa by using sterile sunflower oil, weighed and measured. It was covered with a blanket and then placed under a radiant heater. When the placenta had detached, the mother was asked if she wanted to participate in the study. The nipple and areola zone (in a diameter of ~3 cm) of one breast was treated with AF, an assistant gently wiping the area with the moistened gauze pad. The breasts were not cleaned with water or other solutions. Mothers were randomized to left or right breast application by the closed envelopes method.

Following this treatment, the observer, unaware of which breast had been treated with the AF, entered the room and placed the naked baby in a prone position on the mother's chest, nose to the midline of the mother's sternum, eyes at the level of the nipples. Placing the baby with nose to the midline allowed the baby to sample olfactory signals from both sides of the mother's chest. The baby was covered with a blanket. Observation and recording of the baby's behavior began at that time (6–23 min after birth; median 12.5 min) (Table 1), and continued until it found the nipple and began to suck vigorously. This testing procedure was adopted because our previous research using similar methods demonstrated that spontaneous nipple choice by newborn infant is influenced by breast odor (17). The following behavioral measures were obtained: head turn (both eyes turned to one side of the midline); initial head turn (the first head turn by the baby after initial placement by the observer); hand-to-mouth contact (contact of fist or fingers with lips, > 2 sec); onset of active rooting (first opening of the mouth together with slight movement of the head towards mother's skin); first mouth-areola contact (first time that the baby's lips touch the mother's areola or nipple); sucking latency (age of the baby when nipple sucking was first observed).

Prior to beginning the observation, the mother was told that she and the observer would together watch the baby's behavior to see whether it could find the nipple without any help. Mothers lay on their backs on the delivery bed, lower body covered with a blanket. They were asked to put their hands on the small blanket covering the baby to prevent the baby from falling. The mothers were instructed not to tell the observer which breast had been treated and not to guide, nor help the baby with her hands. The mothers followed the instructions carefully. If they showed some concern about the baby's behavior they discussed it with the observer and continued to remain calm. Oral informed consent was obtained from all mothers, and fathers when present, and the study was approved by the local Ethics Committee.

Results

Natural breast odor vs breast odor plus amniotic fluid

One boy did not respond to the mother's breast during the observation session. Although he displayed normal prefeeding behaviour (onset of rooting at 30 min, hand-to-mouth movement at 41 min), he did not move to the breast, but stayed in the middle of the mother's chest, sucking his thumb. His mother had extremely large breasts and the long distance to the nipples might have hampered the baby's ability to perceive the odor. This baby was not included in the data analysis.

Each of the remaining 30 infants developed the expected prefeeding behavior (Table 1) and made their choice between the treated / untreated breasts. Twenty-two of these 30 babies spontaneously grasped a nipple and began to suck at a median of 46.5 min after birth. The remaining eight babies had mouth contact with one of the nipples (median of first mouth-areola contact = 40 min; range 21–85 min), and repeatedly attempted to grasp it (> 7 attempts) before the observer assisted by placing the nipple in the infant's mouth (median = 47.5 min postpartum). The observer assisted only if the baby appeared unable to grasp the nipple after several minutes of mouth-areola/nipple contact and the mother voiced some concern (median = 5 min, compared to a median of 1 min between first areola contact and sucking for the unassisted babies). None of these infants had attempted to grasp the alternative nipple, and they were therefore scored as having selected the nipple that they repeatedly attempted to grasp. Inability to grasp the nipple appeared to depend on the anatomy of the breasts and nipples, with assistance required for large, soft breasts and flat nipples.

As given in Table 1, 23 babies selected the breast treated with AF, while only 7 babies chose the naturally smelling breast ($p < 0.001$, Fisher's exact test). Of the eight babies who needed assistance to grasp the nipple, six preferred AF and two showed a preference for the natural odor; 17 of 22 babies who successfully grasped a nipple without help chose the AF nipple ($p < 0.001$, Fisher's exact test). After being placed in the midline, 21 babies examined both sides of the mother's chest alternatively and then made their choice—16 grasped the AF breast and 5 the natural odor breast. Nine babies turned only once from midline to the mother's right breast and stayed so. Three babies chose this breast for sucking—all three breasts were AF treated. Six babies moved the head backwards (see below) without turning around and attached to the contralateral breast. Four of these six breasts were AF treated. So even in these nine cases where the babies turned the head only once there seemed to be an active choice of side. Altogether 12 babies adopted this backward approach when they made their final choice, away from the breast most proximal to the nose, until the mouth had passed the areola-nipple area of the contralateral breast and then attached. Eight babies who chose the AF breast and four who chose the natural odor breast showed this unexpected motor behavior.

Out of the seven infants whose body was wiped off to moisten the pad for AF collection, five went to the AF-treated breast while two selected the naturally smelling breast. Among the babies tested with AF collected in a receptacle, 18 of 23 chose the AF-treated breast ($p < 0.005$; binomial test). The proportion of neonates who selected the AF-treated breast was not significantly different between these two subgroups. Moreover, there were no statistically reliable differences in the prefeeding behavior of infants who chose the AF versus those selecting the naturally smelling breast (Table 1). Likewise, the relative frequencies of left/right side preferences did not differ significantly, nor was there a significant directional preference for initial head turning.

Prefeeding behavior and final breast choice were not influenced by mother's age, parity or duration of first or second stage of labour (data not shown).

Effect of amniotic fluid on hand-mouth contact

Hand-mouth contact was observed in only 12 of the 30 babies. This is in sharp contrast to our previous study (17) (babies unwashed when given to the mother), where 27 of 30 babies displayed clear (lasting > 2 sec) hand-mouth contact (chi-square = 14.3; df 1; $p < 0.0002$), (Fig. 1). There also seemed to be qualitative although unquantified differences between the behavior of each of the two groups. Thus, in the present study the babies for a long while seemed uninterested in joining hand to mouth even if they were close to each other. Sucking was elicited only if the hand happened to touch the mouth. In the previous study, in contrast, we observed that if the hand was close to the nares, i.e. 3–4 cm, it was soon put into the mouth and finger sucking began.

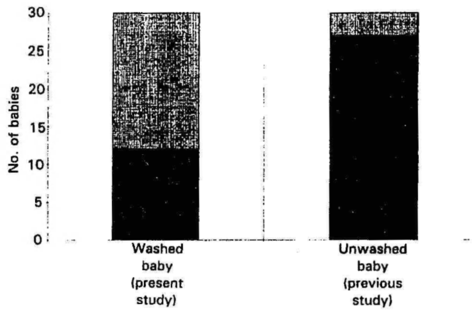

Fig. 1. Number of (washed) babies engaging in hand–mouth contact in the present experiment compared to a previous experiment with unwashed babies (17).

Observations of the prefeeding motor activity

In the first hour after birth, when the baby attempts to reach the nipple it makes use of an extensive repertoire of innate reflexes. For example, intermittent extension and flexion of the lower legs—as in the stepping reflex—were elicited soon after the babies were placed in the prone position on the mother's abdomen. These movements could be observed under the covering blanket; the body pushed upwards in the midline preceded by the appearance of crawling movements of the arms and upper part of the body. The latter type of motor activity culminated shortly before the baby started to move towards the chosen nipple.

It may be of clinical significance that some mothers reported uterine contractions following the baby's leg movements and could also feel that some blood was passed. The observer was able to palpate the contracted womb.

Discussion

Within the first hour after birth, significantly more babies spontaneously selected a breast treated with

AF than the alternative untreated breast. This attraction appears to be based on olfactory cues, since all of the infant subjects sucked from (or repeatedly attempted to grasp) the first nipple with which they had mouth contact. Thus, they effectively chose between the two nipples (AF vs untreated) before initial mouth contact, thereby excluding taste cues. Our results do not necessarily imply that initial nipple attachment and sucking are solely dependent upon olfactory stimuli. Rather, as neonates orient towards distal breast odors they will come into contact with additional cues (e.g. visual, tactile, thermal, taste) that could further facilitate nipple localization and attachment. These cues might have helped the newborn to locate the nipple, but cannot have played a role in the choice between breasts with different smells.

In a previous experiment (17), newborn babies preferred to suck for the first time from an unwashed breast, rather than a washed breast, which indicates that natural breast odors are also attractive. The present data suggest that AF potentiates or overrides the attractiveness of the natural scent of the mother's breast. Positive responsiveness to AF shortly after birth may be a reflection of prenatal experience and familiarization with that substance. As noted in the introduction, investigations with non-human mammals have demonstrated that postnatal responses to odors may have been biased by prenatal exposure to those same scents. Fetal learning is likewise believed to account for the preferences of human neonates for their own mother's voice (1) or the calming influence of rhythmic heart-beat sounds (18, 19).

The biological significance of our findings is unclear, but the presence of familiar stimuli from the intrauterine environment may help the newborns adapt to their novel postnatal existence. Thus, throughout most of the evolutionary history of our species, it was probably common for women to handle their baby during and following delivery, as observed amongst current non-human primates (20). Immediately after parturition, the mother's hands would therefore have been soiled with birth fluids that would likely have been transferred to her breasts when she first attempted to nurse her neonate. In this context, positive responsiveness to the odor of AF would facilitate nipple localization by the neonate in a manner analogous to newborn rodents being guided to the nipple region by birth fluids that their mother had applied to her ventrum (10).

In the present study, where one can assume that the hands of the baby did not smell of AF, the babies showed little interest in joining hand and mouth. This is in contrast to our former study—where the hands smelled of AF (17)—and searching movements were elicited when the hands came close to the nostrils. These observations would further support our suggestion that the odor of AF is attractive and helps the baby find objects to suck.

When newborn babies are placed in the prone position in a cot their motor activity rapidly fades (unpublished observations), in contrast to the behavior of babies placed prone on the mother's chest. It is tempting to speculate that the breast and AF odors, together with other sensory stimulation emanating from the mother, serve as activators of the baby's purposeful, directional motor activity ending in locating the nipple.

The forceful leg movements may have an additional important function, i.e. contributing to uterine contractions. These movements appeared to substitute effectively for uterine massage, or administration of agents contracting the womb, routinely given to avoid accumulation of blood in the uterus. Such early motor activity by the neonate may therefore represent an evolved adaptation that provokes uterine contractions without the need for additional human intervention.

The data presented above illustrate further the salience of maternal odors for newborn infants. Aside from guiding neonates' overt behavioral responses, such olfactory stimuli also appear to have a calming effect on infants and provide a basis for early individual recognition of the mother (14). Therefore, excessive use of products that eliminate or mask natural odor signals (e.g. deodorant, perfume, eau-de-cologne) should perhaps be avoided during the perinatal period.

Acknowledgements.—The study was aided by grants from the Foundation "Frimurare Barnhuset" and from the Karolinska Institute.

References

1. DeCasper AJ, Fifer WP. Of human bonding: newborns prefer their mother's voices. Science 1980;208:1174–6
2. Fifer WP, Moon CM. The role of mother's voice in the organisation of brain function in the newborn. Acta Paediatr 1994; Suppl 397:86–93
3. Smotherman WP, Robinson SR. Psychobiology of fetal experience in the rat. In: Krasnegor NA, Blass EM, Hofer MA, Smotherman WP, editor. perinatal development: a psychobiological perspective. Orlando: Academic Press, pp. 39–60
4. Hepper PG. Adaptive fetal learning: prenatal exposure to garlic affects postnatal preferences. Anim Behav 1988;36:935–6
5. Altbacker V, Hudson R, Bilko A. Rabbit mother's diet influences pups' later food choice. Ethology 1995;99:107–16
6. Hepper PG. The amniotic fluid: an important priming role in kin recognition. Anim Behav 1987;35:1343 6
7. Parfet KAR, Gonyou HW. Attraction of newborn piglets to auditory, visual, olfactory and tactile stimuli. J Anim Sci 1991;69:125 33
8. Schaal B, Orgeur P, Arnould C. Olfactory preferences in newborn lambs: possible influence of prenatal experience. Behaviour 1995;132:351–5
9. Teicher MH, Blass EM. First suckling response of the newborn albino rat: the role of olfaction and amniotic fluid. Science 1977;198:635–6
10. Blass EM, Teicher MH. Suckling. Science 1980;210:15 22
11. Chuah MI, Farbman AI. Developmental anatomy of the olfactory system. In: Doty RL, editor. Handbook of olfaction and gustation. New York: Marcel Dekker, 1995:147–70
12. Logvinenko AV. Recording of fetal respiratory movements based on studying the shifting of the amniotic fluid in the trachea by the Doppler effect. Akush Ginekol 1990;7:38–41

13. Schaal B, Marlier L, Soussignan R. Responsiveness to the odour of amniotic fluid in the human neonate. Biol Neonate 1995;67:397–406
14. Porter RH. Human reproduction and the mother–infant relationship: the role of odors. In: Getchell DV, Doty RL, Bartoshuk LM, Snow JB, Jr, editors Smell and taste in health and disease. New York:Raven Press, 1994: 429–42
15. Makin JW, Porter RH. Attractiveness of lactating females' breast odors to neonates. Child Devel 1989;60:803–10
16. Widström AM, Ransjö- Arvidson AB, Christensson K, Matthiesen A-S, Winberg J, Uvnäs-Moberg K. Gastric suction in healthy newborn infants. Acta Paediatr Scand 1987:76 :566–72
17. Varendi H, Porter RH, Winberg J. Does the newborn baby find the nipple by smell? Lancet 1994;344:989–90
18. Salk L. The effects of the normal heart beat sound on the behavior of the new-born infant:implications for mental health. World Mental Health 1960;12:168–75
19. Salk L. Mother's heart beat as an imprinting stimulus. Transact New York Acad Sci 1962;24:753–63
20. Coe L. Psychobiology of maternal behavior in nonhuman primates. In:Krasnegor NA, Bridges RS, editors, Mammalian Parenting. Biochemical, neurobiological and behavioral determinants. New York:Oxford University Press, 1990:157–83

Received Oct. 11, 1995. Accepted in revised form May 31, 1996

III

Varendi H., Porter R. H., Winberg J.
Natural odour preferences of newborn infants change over time.
Acta Paediatrica 1997; 86: 985–990.

Acta Pædiatr 86: 985–90. 1997

Natural odour preferences of newborn infants change over time

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Varendi H, Porter RH, Winberg J. Natural odour preferences of newborn infants change over time. Acta Pædiatr 1997; 86: 985–90. Stockholm. ISSN 0803–5253

At their first sucking contact, neonates prefer an unwashed breast to a washed one, but an amniotic fluid (AF)- treated breast over a "natural odour" breast. We examined the development of these neonatal olfactory preferences. On days 3–4 significantly more babies still selected their mother's unwashed breast ($n = 21$) than the washed alternative ($n = 8$). Preferences for natural breast odours were more pronounced for girls than boys. In a subsequent experiment comprising another 28 babies, the number of babies who selected a naturally scented ($n = 9$) vs an AF-treated breast ($n = 19$) on days 2–5 were not reliably different. However, babies who selected the natural breast had longer pre-test maternal contact and had spent more time breastfeeding. Ten babies who chose the AF breast in the latter experiment were tested in the same manner several days later; all preferred the naturally smelling breast. While preferences for AF fade after birth, responsiveness to natural breast odours may be enhanced by postnatal experience. □ *Amniotic fluid, breast odour, neonatal behaviour, olfaction, postnatal learning*

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Natural maternal odours help guide the newborn to the nipple for the initial breastfeeding attempt (1). Within the first hour after birth, significantly more babies selected their mother's naturally smelling breast rather than the alternative breast that had been thoroughly washed to reduce its characteristic odour. Moreover, at 2 weeks of age, bottle-fed infants responded preferentially to a cotton pad impregnated with the breast odour of an unfamiliar lactating woman over pads bearing that same female's axillary odour (2) or the scent of their own familiar formula (3).

Neonates also respond positively to the odour of amniotic fluid (4). In a recent experiment, babies preferred to suck for the first time from their mother's breast that had been treated with a small amount of amniotic fluid rather than the alternative untreated control breast (5). Thus, within the first hour after birth, the odour of amniotic fluid appears to enhance the attractiveness of olfactory cues emanating from the mother's breasts, or possibly override those scents. Because babies in the latter study were bathed and dried immediately after birth and testing began when they were only 6–23 min. old, they had little opportunity to become familiar with the odour of AF postnatally. Therefore, it is likely that the observed positive responsiveness to AF reflects fetal familiarization with that substance (i.e. prenatal olfactory learning) rather than postnatal exposure to AF. This conclusion is supported by animal studies demonstrating that neonates recognize the odour of their own amniotic fluid (6) as well as the scent of substances that their mother had ingested while pregnant with the subject offspring (7).

Related studies have documented that infants' responses to specific odourants may also vary as a function of their experience with those scents following birth. For example, mere exposure to an artificial scent within the first 2 days postpartum results in preferential orientation to that same stimulus at 2 weeks of age (8). Breastfed babies likewise learn the odour of their mother's perfume (9) and rapidly develop the ability to discriminate between the individual odour signature of their own mother versus that of other lactating women (10, 11).

In the present series of experiments, we investigated the temporal development of neonates' responsiveness to amniotic fluid and breast odours over the first several days following birth. Because exposure to AF diminishes abruptly beginning at birth, whereas breastfeeding infants have recurring exposure to odours emanating from their mother's nipple/areola region, it was hypothesized that the relative salience or attractiveness of odours from these two sources would not remain constant during the first 1–2 weeks of postnatal existence.

Spontaneous nipple localization and pre-feeding behavior by the newborn baby have been described during the first hours of postnatal life (12, 13). Likewise, the role of naturally occurring odours in attracting the baby to the nipple has been documented only within the first 90-min. after birth (1). The present experiment assessed whether the sequential pattern of pre-feeding behavior of the newborn remains similar after regular breastfeeding has been established and whether maternal breast odours may continue to help guide infants to the nipple several days after birth.

Materials and methods

Experiment 1: Pre-feeding behaviour and responses to natural breast odours at postnatal days 3–4

Subjects and questionnaires. Twenty-nine healthy term newborns and their healthy, non-smoking mothers were studied. After uncomplicated vaginal delivery without analgesia (routine in Tartu), the mothers were asked to participate in the study and to keep a diary, i.e. to record the time at the beginning and end of the following activities:

- all breastfeeding bouts (nose–breast contact),
- periods of close contact with the baby (holding the baby in her arms or staying together in the mother's bed; including breastfeeding sessions),
- periods the baby spent in the nursery.

Participating mothers also provided information concerning the use of perfumes, deodorants, shampoos, soaps or other scented products, as well as painful or sore nipples and the application of ointments to the breasts or nipples.

The coded questionnaires were analysed separately from the results of the tests with the babies. The number of breastfeeding bouts, total duration of breastfeeding and the cumulative duration of close contact with the mother were calculated for the total postnatal time period preceding testing.

The study was approved by the Ethics Committee at Tartu University and the mother's informed consent was obtained.

Testing procedure. Testing occurred on postnatal days 3–4 (mean age 67 ± 10 h) when the babies had spontaneously woken (≥ 2 h since the previous breastfeeding bout). One of the mother's breasts was washed by a nurse with an odourless liquid soap (Mildtval) from the chest's midline to the lateral, upper and lower margin of the breast's soft tissue, to remove the natural smell, then rinsed with a wet towel and dried. Choice of the breast to be washed was random (closed envelope method); for 15 mothers the left breast was washed, and in 14 cases the right breast.

The observer, unaware of which breast had been washed, entered the room and placed the naked baby in a prone position on the mother's bare chest, nose to the midline of her sternum, eyes on the level of the nipples. From this position, the baby could presumably sample olfactory cues from both sides of the mother's chest. The baby was covered with a small cotton cloth. Observations and recording of the baby's behaviour began at that time and continued until she/he displayed a clear nipple choice—i.e. grasped the nipple and began to suck. Mothers remained lying on their back during the test and were instructed to place their hands on the small blanket covering the baby to prevent the infant from falling—but were asked not to otherwise guide nor help the baby.

Experiment 2A: Days 2–5

Subjects. A new cohort of 28 mothers along with their newborn infants was selected and recruited as described in Exp. 1 (see Table 3 for demographic details). The Ethics Committee approved the study.

Testing procedure. After delivery, 2–4 sterile gauze pads (7 × 7 cm) were moistened with the mother's own amniotic fluid (AF) collected in a receptacle. The pads were sealed in two different plastic bags and frozen.

Exp. 2A was conducted on postnatal days 2–5 (age range 43.5–105 h). The frozen AF pad(s) in one bag were thawed by placing the sealed plastic bag in a water bath (35–37°C) for 30 min before the start of the test. The test trial began at least 2 h following the preceding breastfeeding bout, after the baby had spontaneously woken. Immediately before the test trial began, the nipple and areola region (diameter of approximately 3 cm) of one of the mother's breasts was treated with AF by gently wiping the area with the moist (thawed) gauze pad. The alternative breast remained untreated and neither breast was cleaned with water or other solutions. Mothers were randomly assigned to left ($n = 14$) or right ($n = 14$) breast treatment by the closed envelope method. The baby was then placed naked on the mother's bare chest, with further details of the testing procedure the same as described for Exp. 1.

Experiment 2B: Days 5–10

Because babies in the preceding experiment who selected the natural breast had spent more time breastfeeeding and in contact with their mother than did those choosing the AF-treated breast (see Results section 2A), we hypothesized that the latter babies might show a reversed preference for the natural breast odour following increased breastfeeding experience.

Subjects. To assess this hypothesis, 10 of the 19 babies (6M,4F) who sucked from the AF-treated breast on postnatal days 2–5 (Exp. 2A) were recruited to participate in an additional test. These 10 babies were selected for practical reasons, i.e. they all lived in Tartu and were therefore readily available for the follow-up test. These infants and their mothers were discharged from the maternity ward on postnatal days 3–5. The observer visited them at their homes after a mean interval of 96.5 ± 16.3 h had elapsed since their participation in the previous experiment (2A). At the time of testing for Exp. 2B, babies' postnatal ages ranged from 102–236 h (mean = 165 ± 26).

Testing procedures. A second pad that had been moistened with the baby's AF and frozen immediately after birth was thawed in lukewarm water approximately 30 min before the start of the test session. One breast of the baby's mother was treated with AF and the alternative breast remained untreated; the baby was tested for

Table 1. Comparison of pre-feeding behaviour on postnatal days 3–4 (Exp. 1) and during the first postnatal 90 min (1). (Latency measures calculated from the beginning of the observation).

	Postnatal 90 min (n = 30)	Postnatal days 3–4 (n = 29)
Onset of active rooting (min)		
Median	20	0.17***
Range	10–41	0.08–3
Hand–mouth contact (min)	(n = 27/30)	(n = 8/29)***
Median	23	0.5***
Range	7–60	0.2–1
First mouth–nipple contact (min)		
Median	51.5	2***
Range	22–100	0.5–13
Sucking latency (min)		
Median	52.5	2***
Range	22–100	0.5–13

*** $p < 0.001$.
For the comparison between the proportions of babies showing hand–mouth contact, $\chi^2 = 23.8$, df = 1. For all other comparisons the Mann–Whitney U-test was used.

its spontaneous choice between the two breasts as in Exp. 2A. For each mother–baby pair, the breast treated with AF was the same as the AF-treated breast in the previous experiment. For five babies, the mother's right breast was treated with AF, whereas AF was applied to the left breast for the remaining five infants. Further procedural details were the same as those described for Exp. 2A.

Results

Experiment 1

Age-related differences in pre-feeding behaviour. The sequence of pre-feeding behaviour of the babies in the present experiment (on postnatal days 3–4) was similar to that previously reported for the first postnatal hour (1); i.e. crawling, active rooting, hand–mouth contact (although less frequent), mouth–nipple contact, sucking. However, the initiation of the different activities occurred much earlier in the older babies, where the whole behavioural sequence, concluding with nipple contact and the initiation of sucking, was completed in 2 min as compared to 52.5 min in the newborn infants (Table 1). There were no differences in the pre-feeding behaviour between boys and girls.

Responses to breast odours. Twenty-one of the 29 babies chose the unwashed, naturally smelling breast, whereas 8 went to the washed breast (binomial test: $z = 2.23$; $p < 0.03$; 2-tailed; Table 2, Fig.1). In one of the latter cases, the mother had sore, cracked nipples and treated her unwashed breast (but not the washed breast) with ointment following the preceding feeding bout. The ointment could have been aversive to the baby and thereby influenced the breast-choice. In an additional case where the baby selected the washed breast, there was a drop of milk coming from the nipple after the washing procedure.

There was a statistically significant sex difference in breast choice—all of the girls ($n = 11$) selected the unwashed breast, whereas 10 boys chose the unwashed breast and 8 went to the washed breast (comparing proportion of boys vs girls who chose the unwashed breast: $\chi^2 = 6.40$, df = 1; $p < 0.02$). This sex difference persisted ($p < 0.03$) even if the two boys whose choice might have been biased by the experimental conditions (ointment and leakage of milk, see above) were excluded from the calculations. In other respects there were no differences between those who chose the washed vs the unwashed breast (Table 2).

Experiment 2A

All 28 babies displayed a clear choice (AF vs untreated breast) by grasping a nipple and beginning to suck vigorously.

Table 2. Comparisons between babies who chose the unwashed vs washed breast for sucking on postnatal days 3–4. Data presented as medians (range).

	Choice of unwashed breast (n = 21)[a]	Choice of washed breast (n = 8)[a]
Girls/boys	11/10	0/8*
Birthweight (g) (mean ± SD)	3614 ± 484	3650 ± 420
Age at first breastfeeding (min)	40 (20–885)	35 (15–60)
No. of breastfeedings before testing[b]	17 (6–33)	16 (8–32)
Cumulative breastfeeding time (min)[b]	310 (105–560)	330 (90–560)
Duration of mother–infant close contact (h)[b]	23 (8–70)	31 (16–50)
Age at experiment (h)	68 (55–74)	59 (51–94)
Time to first mouth–nipple contact (min)	2 (0.5–8.5)	1.5 (0.8–13)
Washed left/right breast	12 L/9 R	3 L/5 R
Choice of left/right breast	9 L/12 R	3 L/5 R

[a]There were no differences in mother's age, parity, duration of 1st or 2nd stage of labour (data not shown) between groups who chose unwashed or washed breast for sucking.
*$p < 0.02$, χ^2.
[b]Prior to testing.

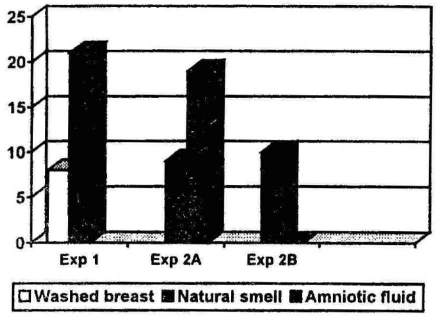

Fig. 1. Comparison of choice of breast for sucking in Exp. 1, Exp. 2A and Exp. 2B. Exp. 1: washed breast vs unwashed breast (8 vs 21, $p < 0.03$) at a mean postnatal age of 67 ± 10 h. Exp. 2A: Amniotic fluid treated breast vs natural odour breast (19 vs 9, n.s.) at a mean postnatal age of 68 ± 15 h. Exp. 2B: Amniotic fluid treated breast vs natural odour breast (0 vs 10, $p < 0.02$) at a mean postnatal age of 165 ± 26 h.

Nineteen of the babies chose the AF- treated breast and 9 the naturally smelling (untreated) breast for sucking (binomial test: $z = 1.70$, $p < 0.09$; Table 3, Fig. 1). Babies who selected the untreated breast had longer cumulative duration of breastfeeding and close mother–infant contact prior to the test than did babies who sucked from the AF-treated breast (Table 3). There were no additional significant differences between these two subgroups neither for other variables given in Table 3 nor for mother's age, parity, duration of 1st or 2nd stage of labour (data not shown).

Experiment 2B

All 10 babies chose the naturally smelling (untreated) breast, rather than the AF-treated breast, for sucking (Fig.1), which was significantly greater than the number expected by chance alone ($p < 0.002$, binomial test). There were no reliable differences observed in the pre-feeding behaviour compared to Exps 1 or 2A.

Discussion

Experiment 1

Age-related differences in pre-feeding behaviour. The pattern of pre-feeding behaviour observed in Exp. 1 (on postnatal days 3–4) was similar to that previously recorded during the first postnatal hour. The less frequent occurrence of hand–mouth contact by the infants tested on days 3–4 is most likely due to the absence of AF on their hands, whereas AF was still present on the hands of neonates observed during the first 90 min. This morphological stability from birth to day 4 suggests that there may be an innate program for the infants' early feeding behaviour. The general motor inactivity during the first 20 min after birth might indicate immediate postnatal exhaustion, which contrasts with the alertness and heightened motor activity at 60–90 min postpartum. Differential physiological conditions may have contributed to the dramatic differences in latencies of the pre-feeding behaviour pattern across the two age groups. The more rapid responses of the older babies are also likely to be the result of improved motor performance and prior breastfeeding experience (compared to the younger babies who were observed during their initial feeding attempts and therefore had no pretest contact with the nipple). Possible neuronal mechanisms underlying the difference in tempo in localizing the nipple will be examined within the General Discussion section.

Washed vs unwashed breast. The significant preference for the unwashed, rather than the washed, breast indicates that natural odours continue to aid nipple localization and sucking 3–4 days after birth. Thus, the contribution of maternal breast odours to effective breastfeeding is not limited solely to the immediate postpartum period, as described earlier (1). In related research, infants were

Table 3. Results of choice tests with a breast treated with AF vs an untreated (naturally smelling) breast on postnatal days 2–5 (Exp. 2A), and characteristics of babies in each response category. Data expressed as medians (range).

	Choice of AF-treated breast ($n = 19$)	Choice of untreated breast ($n = 9$)
Girls/boys	8/11	5/4
Birthweight (g) (mean ± SD)	3710 ± 389	3841 ± 322
No. of breastfeedings before testing[a]	19 (13–45)	26 (14–36)
Cumulative breastfeeding time (min)[a]	375 (200–605)	490 (235–820)*
Total time spent in the nursery (h)[a]	7 (0–82)	7 (0-14)
Duration of mother–infant close contact (h)[a]	25 (9–51)	38.5 (24–66)*
Age at experiment (h)	69 (44–105)	74 (50–92)
Time to first mouth-nipple contact (min)	2 (0.2–10)	2.5 (0.5–13)
AF applied on left/right breast	7 L/12 R	7 L/2 R
Choice of left/right breast	7 L/12 R	2 L/7 R

*$p < 0.05$, Mann–Whitney U-test.
[a]Prior to testing.

found to orient preferentially to a stimulus pad carrying the breast odour of a lactating female when paired with either a control odour or a clean (non-odourized) pad (2). The most parsimonious conclusion would seem to be that these neonates were attracted to the breast odour rather than displaying avoidance of both the control odour and the clean stimulus pad. Such positive responsiveness to maternal breast odours is also likely to account for the preference for the unwashed breast observed in Exp. 1.

The choice of the unwashed breast was more pronounced for the girls than for the boys. In our previous experiment with similar testing procedures, performed within the first 90 min after birth, both boys and girls preferred the unwashed breast (1). Moreover, 2-week-old bottle-fed boys, as well as girls, oriented preferentially towards the odour of a breast pad from an unfamiliar lactating female rather than a pad treated with their familiar formula (3). Thus, immediately after birth and at 2 weeks of age, boys are capable of perceiving maternal breast odours and respond positively to such cues. Nonetheless, the sex differences observed in Exp. 1 are corroborated by several related studies in which infant boys were likewise less responsive than girls to maternal and artificial odours (2, 14). Overall, these data suggest tentatively that baby boys may have a less acute sense of smell than girls. This would be consistent with various studies documenting superior performance by females, ranging in age from 5 to 90+ y, on a variety of olfactory discrimination/recognition tasks (15–17). Of course, it is also possible that additional factors, such as differences in motivation, distractibility, sensitivity to non-olfactory cues and olfactory learning could account for the disparities between boys and girls in our experiment.

Relative attractiveness of AF vs natural breast odour. As mentioned above, when tested immediately after birth, significantly more babies spontaneously chose to suck from their mother's breast that had been treated with amniotic fluid (presumed to have been a salient olfactory feature of their intrauterine environment) as compared to the alternative untreated breast (5). The following series of experiments was an attempt to determine whether this relative preference for AF over natural breast odours would remain stable or show a reversal as the baby became older and had increasing exposure to maternal breast odours in the context of nursing.

Experiment 2

During the initial tests (at days 2–5), more babies chose to feed from the breast treated with AF than from the naturally smelling breast, however this difference was not statistically significant. The reliable relative preference for an AF-treated breast that had been previously observed within the first 90 min after birth (5), was therefore not evident at this later age. As seen in Table 3, babies who selected the untreated (naturally smelling) breast had spent more time breastfeeding and in close contact with their mother than had those infants who selected the AF-treated breast. There was no reliable difference in the ages of infants in these sub-groups at the time of testing. These data indicate that the relative attractiveness of natural breast odours increases as a function of breastfeeding experience and maternal contact—rather than age *per se*.

The latter hypothesis is further supported by the results of Exp. 2B. That is, 10 infants who had selected the AF- treated breast on days 2–5, all displayed reversed preferences for the naturally smelling breast several days later. Thus, babies learn to modify their responsiveness to biologically meaningful odours as a consequence of prior exposure and experience with those cues. At birth, neonates display a preference for odours that were an important feature of their prenatal environment (AF). During the next several days, AF odours are no longer present but breastfed infants have repeated exposure to maternal breast odours associated with food intake and tactile stimulation provided by their mother. In an adaptive manner, the effects of postnatal olfactory learning gradually begin to predominate over earlier odour preferences arising from prenatal experience.

It should be pointed out that postnatal learning is not the sole factor involved in the mediation of infants' attraction to natural breast odours emanating from lactating females. As mentioned above, maternal breast odours help guide the newborn to the nipple for the initial feeding (1) and 2-week-old infants that had been exclusively bottle fed since birth orient discriminatively to such scents emanating from an unfamiliar lactating female (2, 3). Thus, such early attraction to breast odours appears to be independent of postnatal learning since it is demonstrated by infants who have had no exposure to those specific cues after birth. Nonetheless, our data indicate that positive responsiveness to maternal breast odours may be augmented by postnatal experience with those same scents.

On the other hand, the observed heightened (relative) preference for maternal breast odour over AF with increasing age and experience may also reflect reduced attractiveness (possibly even aversion) of that substance.

General discussion

Nipple attachment and sucking by mammalian neonates must function effectively within hours after birth. Newborn mammals play an active role in the feeding process. Despite wide interspecies differences in the degree and form of maternal involvement in infants' feeding behavior, newborn young (including humans) display a similar series of complex motor activities that result in nipple localization and milk ingestion. There was an impressive reduction of the median latency to sucking from 52.5 min immediately after birth to 2 min at 3–4 days postpartum. The presence of complex pre-feeding behaviour at birth, partly triggered by specific odour(s), suggests that the underlying neuronal networks that can generate specific muscular activity, producing vital behaviours, had already been formed prenatally. Ensuing motor development results from the interaction between such genetically influenced programs and environmental input. According to the theory of "neuronal group selection", "neuronal Darwinism" (18) or "selective stabilization of developing synapses" (19), networks that are used in an adaptive manner become strengthened, while those that serve no function are discarded, as maturation proceeds. Through this process of selection only well functioning networks will persist, and these may continue to be further refined by environmental influences. Such a process might explain the difference in tempo of the pre-feeding behaviour at 90 min compared to 3–4 days.

Natural odours produced by the mother may be an important factor for the elicitation of the sequence of pre-feeding behaviour and effective sucking. Human infants have well-developed olfactory discrimination abilities. Newborn babies are attracted by the mother's natural breast odours during their initial postnatal contact (1). It is reasonable to assume that the origin of this preference may be a genetically mediated programming of the newborn infant's brain to respond to this special odour. Alternatively, if foetal olfactory learning occurs, as suggested by earlier demonstrations that the smell of AF elicits positive responses by neonates (4, 5), the odorous compounds that make AF attractive might also be produced by glands in the areola region (20). Accordingly, at birth, breast odours may be attractive because they overlap to some extent with odours previously experienced by the foetus. Despite this hypothesized resemblance between AF and breast odours, these two odours are distinguishable and the familiar AF is initially preferred. However, with increasing exposure to breast odours, and concomitant elimination of AF, infants gradually develop a preference for the natural breast odour over AF. Exposure to breast odours in the reinforcing context of feeding, as well as reduced attractiveness of AF, may further contribute to this reversal in early olfactory preferences.

As discussed above, the discriminative responsiveness to maternal breast odours by girls, but not boys, in Exp. 1 is consistent with previous reports of sex differences in olfactory mediated behaviour in neonates. It should be emphasized, however, that such sex differences in infants' responses to olfactory cues have often not been observed, as in our second series of experiments. This is an issue clearly in need of further investigation.

Recent physiological research suggests that norepinephrine plays an important role in early olfactory learning (21, 22). Norepinephrine neurons in the locus coeruleus synapse with the olfactory bulbs and are, at least in rats and sheep, a prerequisite for learning specific odour cues. During the first hours after human birth, plasma levels of catecholamines (especially norepinephrine) are increased 20–30 fold as compared to measures from older infants (23). The locus coeruleus is also likely to be highly activated immediately after birth (24). Newborn babies might therefore be physiologically prepared to learn to recognise the olfactory signature of their mother while interacting with her during the first postnatal hours.

Acknowledgements.—The study was aided by grants from the Foundation "Frimurare Barnhuset" and from the Karolinska Institute.

References

1. Varendi H, Porter RH, Winberg J. Does the newborn baby find the nipple by smell? Lancet 1994; 344: 989–90
2. Makin JW, Porter RH. Attractiveness of lactating females' breast odors to neonates. Child development 1989; 60: 803–10
3. Porter RH, Makin JW, Davis LB, Christensen KM. An assessment of the salient olfactory environment of formula-fed infants. Physiol Behav 1991; 50: 907–11
4. Schaal B, Marlier L, Soussignan R. Responsiveness to the odour of amniotic fluid in the human neonate. Biol Neonate 1995; 67: 397–406
5. Varendi H, Porter RH, Winberg J. Attractiveness of amniotic fluid odor: evidence of prenatal learning? Acta Paediatr 1996; 85: 1223–7
6. Hepper PG. The amniotic fluid: an important priming role in kin recognition. Anim Behav 1987; 35: 1343–6
7. Hepper PG. Adaptive fetal learning: Prenatal exposure to garlic affects postnatal preferences. Anim Behav 1988; 36: 935–6
8. Davis L, Porter RH. Persistent effects of early odor exposure on human neonates. Chemical Senses 1991; 6: 169–74
9. Schleidt M, Genzel C. The significance of mother's perfume for infants in the first weeks of their life. Ethol Sociobiol 1990; 11: 145–54
10. Macfarlane A. Olfaction in the development of social preferences in the human neonate. In: Porter R, O'Connor M, editors. Parent–infant interaction (Ciba Found. Symp.33). New York: Elsevier, 1975; 103–13
11. Russell MJ. Human olfactory communication. Nature 1976; 260: 520–2
12. Widström AM, Ransjö-Arvidson AB, Christensson K, Matthiesen A-S, Winberg J, Uvnäs-Moberg K. Gastric suction in healthy newborn infants. Acta Paediatr Scand 1987; 76: 566–72
13. Righard L, Alade MO. Effect of delivery room routines on success of first breast-feed. Lancet 1990; 336: 1105–7
14. Balogh RD, Porter RH. Olfactory preferences resulting from mere exposure in human neonates. Infant Behav Develop 1986; 9: 395–401
15. Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. Smell identification ability: changes with age. Science 1984; 226: 141–3
16. Doty RL, Applebaum S, Zusho H, Settle RG. Sex differences in odor identification ability: a cross-cultural analysis. Psychologia 1985; 23: 667–72
17. Koelega HS, Koster EP. Some experiments on sex differences in odor perception. Ann NY Acad Sci 1974; 237: 234–46
18. Edelman GM. Neural Darwinism: Selection and reentrant signaling in higher brain function. Neuron 1993; 10: 115–25
19. Changeux JP, Danchin A. Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks. Nature 1976; 264: 705–12
20. Stafford M, Horning MG, Zlatkis A. Profiles of volatile metabolites in body fluids. J Chromatograph 1976; 126: 495–502
21. Brennan P, Kaba H, Keverne EB. Olfactory recognition: a simple memory system. Science 1990; 250: 1223–6
22. Sullivan RM, Zyzak DR, Skierkowski P, Wilson DA. The role of olfactory bulb norepinephrine in early olfactory learning. Develop Brain Res 1992; 70: 279–82
23. Lagercrantz H, Slotkin TA. The "stress of being born". Sci Am 1986; 254: 92–102
24. Lagercrantz H. Stress, arousal, and gene activation at birth. News Physiol Sci 1996; 11: 214–8

Received Jan. 10, 1997. Accepted in revised form Apr. 25, 1997

IV

Varendi H., Christensson K., Porter R. H., Winberg J.
Soothing effect of amniotic fluid smell in newborn infants.
Early Human Development 1998; 51: 47–55.

ELSEVIER Early Human Development 51 (1998) 47–55

Early Human Development

Soothing effect of amniotic fluid smell in newborn infants

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Received 6 May 1997; received in revised form 29 July 1997; accepted 29 July 1997

Abstract

Newborn young of several mammalian species are attracted to the odor of amniotic fluid (AF); these chemical cues also appear to calm neonates and help them adapt to their novel postnatal environment. AF odor likewise elicits positive (head orientation) responses by human infants. The present study systematically examined whether the odors of AF and mother's breasts influence the crying of the newborn infant, when separated from its mother. The total crying time from 31–90 min postnatal was registered on tapes in 47 healthy fullterm newborns, allocated to one of three conditions: exposure to either AF or breast odor or no exposure (controls). Babies exposed to AF smell cried significantly less (median 29 s) than babies in the two other groups (breast odor—301 s, controls—135 s). The data are consistent with the hypothesis that the fetus may become familiar with chemical cues present in the intrauterine environment. Our data provide new evidence of the human baby's fine olfactory discrimination capacity, and add to the growing body of evidence indicating that naturally occurring odors play an important role in the mediation of infants' early behavior. © 1998 Elsevier Science Ireland Ltd.

Keywords: Amniotic fluid; Association learning; Crying; Newborn; Smell

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PII S0378-3782(97)00082-0

1. Introduction

In mammals, the sense of smell becomes functionally mature at an early stage of individual development. Anatomical, behavioral and physiological evidence indicates that the olfactory system of some species may be functional in utero [1]. The adaptive significance of this precocial maturation of olfaction is illustrated by the reliance of neonatal rodents on maternal odors for orientation to the nest area [2,3], as well as the critical importance of such chemical cues for effective nipple localisation and the initiation of sucking by newborn rats [4] and rabbits [5]. Biologically relevant odors are also salient for newborn humans. Beginning within the first hour following birth, babies display an inborn attraction to maternal breast odors [6,7]. Moreover, newborn infants respond positively to the odor of their own amniotic fluid (AF) [8,9], which may be a reflection of intrauterine olfactory learning, as documented in nonhuman animals [10,11]. Although the biological and clinical relevance of babies' responsiveness to AF odor is not understood at present, it has been suggested that prenatal familiarisation with odors that are likely to continue to be encountered immediately after birth may help the baby adapt to the otherwise novel postnatal environment [8,9]

Animal experiments have further shown that exposure to familiar maternal odors results in reduced rates of locomotor activity and ultrasonic distress calls by isolated neonates [12,13]. Human babies that are separated from their mother at birth likewise emit distress cries that lessen abruptly when maternal bodily contact is reinstated [14,15]. Although the sensory modalities involved in this maternal-contact effect were not investigated in the latter studies, preliminary observations suggest that crying by 1–2 day old infants may decrease when they are presented with the odor of their mother's hospital gown [16]. Based upon the research reviewed above, the present experiment was a systematic attempt to assess the effects of the odors of AF and the mother's breasts on crying behavior of newborn infants.

2. Methods

2.1. Subjects

Sixty healthy fullterm newborns born of healthy, non-smoking mothers with normal pregnancies and vaginal deliveries without analgesia were recruited for this study. Clinical data are given in Table 1. Mothers of participating infants gave their oral informed consent during the early stage of labor and the study was approved by the local research ethics committee.

2.2. Postnatal treatment

Immediately after birth the baby's mouth and pharynx were aspirated and the umbilical cord clamped and cut. The baby was bathed, dried, weighed and measured according to standard hospital routines and a surface electrode (C 1800K, Anritsu, Japan) of a thermometer (52 K/J, Fluke, Litho, USA) was taped to the neonate's

Table 1
Clinical characteristics of the study groups

	AF ($n=16$)	B ($n=15$)	C ($n=16$)
Boys/girls	10/6	7/8	8/8
Birth weight (g)	3838±467	3778±433	3661±340
Mothers age (years)	21.4±3.4	24.9±4.7	25.6±5.5
Primiparas/multiparas	9/7	4/11	6/10
Duration of			
I stage of labor (h)	8.1±3.7	6.0±3.0	8.6±2.4
II stage of labor (min)	31±24	18.6±19	22.8±14.5
Baby's axillary temperature at 90 min (°C)	36.7±0.4	37.0±0.2	37.0±0.3

Data are expressed as mean±S.D..
No significant differences between any of the groups.

axilla. The baby was wrapped in cotton clothes (napkins) and blanket, and placed on an examination table under a radiant heater (Fig. 1). The temperature in the delivery room was 22–26°C, the air temperature measured at the level of the infant's cheek during the period of observation was 25–27°C.

Within 16–30 min after birth, a portable stereo cassette-corder (Sony TCS-430) was placed ~20 cm from the baby's head and switched on to register audible crying. Babies stayed in the same position until the end of the observation period (i.e. 90 min postnatal). At that time, the axillary temperature was recorded, the thermometer

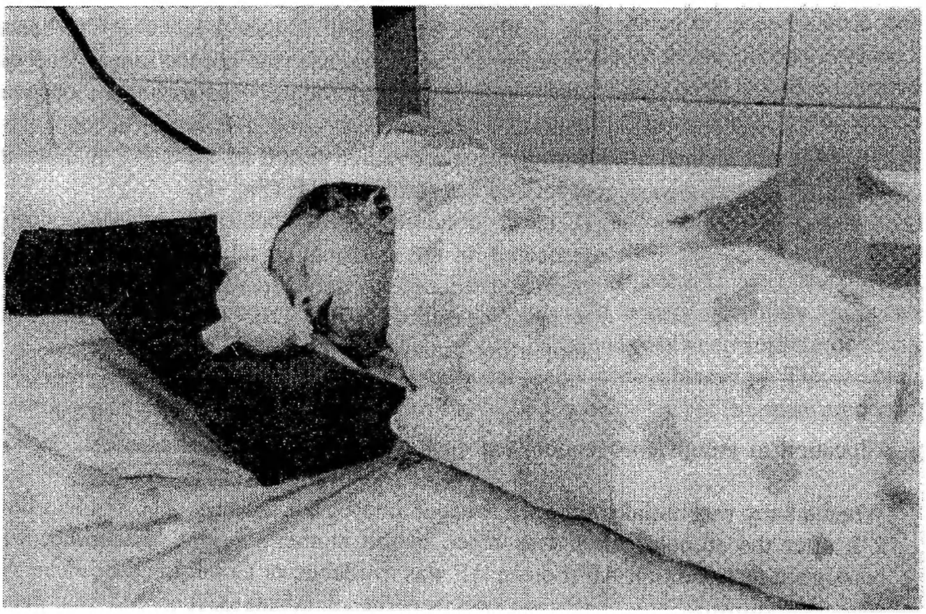

Fig. 1. Study position: baby, wrapped in cotton cloths and blanket, under a radiant heater, exposed to treated cotton cloth, cassette-corder placed ~20 cm from the baby's head to register crying.

electrode removed, and the baby given to the mother for the first time for the initial breastfeeding session. All babies were tested in the same delivery room. Among the five midwives recruited for the testing procedures, the one on duty at the delivery was responsible for the recording. Each of the midwives recorded crying by babies in all three treatment conditions. The mother's name and age of the baby at start of recording were recorded on the tape and the name and date were also written on the tape cassette and protocol log.

2.3. Treatment conditions

Each baby was assigned to one of three treatment conditions:

AF	(AF odor). A sterile cotton cloth ($\sim 7 \times 7$ cm) was moistened with the baby's own AF that had been collected in a receptacle at delivery. This cloth was attached to cover a muzzle of a plastic funnel ($\sim 8 \times 8$ cm) that was placed approximately 2 cm from the baby's nose. The cloth/funnel remained in this position throughout the observation period, and never came into direct physical contact with the baby.
B	(Breast odor). At least 2 h prior to delivery, a cotton cloth (30×15 cm) was taped directly over one side of the mother's bare chest so that it covered one breast including the areola region (but not the nipple) and the corresponding axillary region. This stimulus cloth was removed after delivery and presented to the baby during the observation period in the same manner as described for condition AF (Fig. 1).
C	(Control condition). The babies in this condition remained on the examination table with no additional treatment throughout the observation period (i.e. they were not exposed to either an odor stimulus or the supporting funnel).

Allocation to groups was random except for the following circumstances:

When a baby was initially assigned to Condition B, but delivery occurred less than 2 h after the stimulus cloth was taped to the mother's breast, that infant was reassigned to Condition AF if clean AF was available, or Condition C if clean AF was not available. Such reallocation occurred in seven babies. It should be pointed out that none of the babies was crying when first placed in the testing situation (i.e. before data collection began).

Because of technical problems, it was not possible to record crying by several of the babies during the postnatal period of 16–30 min. Data analysis was therefore restricted to a 60-min period, i.e., 31–90 min after birth. From the original population of 60 babies, 13 babies for whom complete crying data were not available throughout this 60-min observation period were excluded: five each from conditions AF and B, and three from condition C. In five of these cases, the mother decided to breastfeed her baby before the end of the observation period, whereas technical problems were the cause of missing data for the remaining eight babies. This resulted in the following number of babies in the three conditions: AF = 16; B = 15; C = 16.

2.4. Analyses of taped cries

The final analysis of all tapes was done by a single observer who was not aware of the condition to which individual babies had been assigned. Each baby's total crying duration was measured in seconds per standardised 15-min periods relative to the time of birth (e.g. 31–45 postnatal min; 46–60 postnatal min, etc.). The analyses were done "by hand" with two stopwatches; one for measuring 15-min periods, another for recording crying duration.

Before the analyses of the tapes began, KC—who had previous experience with similar crying measures [14]—and HV defined crying so that it could be distinguished from other briefer, more quiet noises, such as whimperings. This same procedure was then repeated by HV and the observer. Reliability was assessed by comparing crying duration judgements between these two investigators (inter-rater reliability) and across repeated (two) judgements by the single observer (intra-rater reliability) for five 5-min periods during which a baby cried. No significant differences were found for these crying duration comparisons. The mean between-observer variation was 1.1 s per 5-min period and the mean within-observer variability = 0.9 s The calculated mean within-observer error was 1.0±0.1% according to the measured crying duration and between-observer error 1.2±0.3%.

2.5. Statistical analysis

Nonparametric tests (Kruskall–Wallis and Mann–Whitney U test) were used to assess differences between treatment conditions. To assess reliability, tests were calculated as $\sqrt{(\Sigma d^2)/(2n)}$, where "d" is the difference between repeated measurements in one observer or the measurements by two observers. The errors of measurements were calculated as $(dn/\Sigma m) \times 100$, where "m" is the measured crying time (s), and were expressed as mean±S.D..

3. Results

Median cumulative crying times during the entire 60-min observation period (postnatal min 31–90) for the three treatment conditions are shown in Fig. 2. An overall statistical comparison using the Kruskall–Wallis test revealed reliable

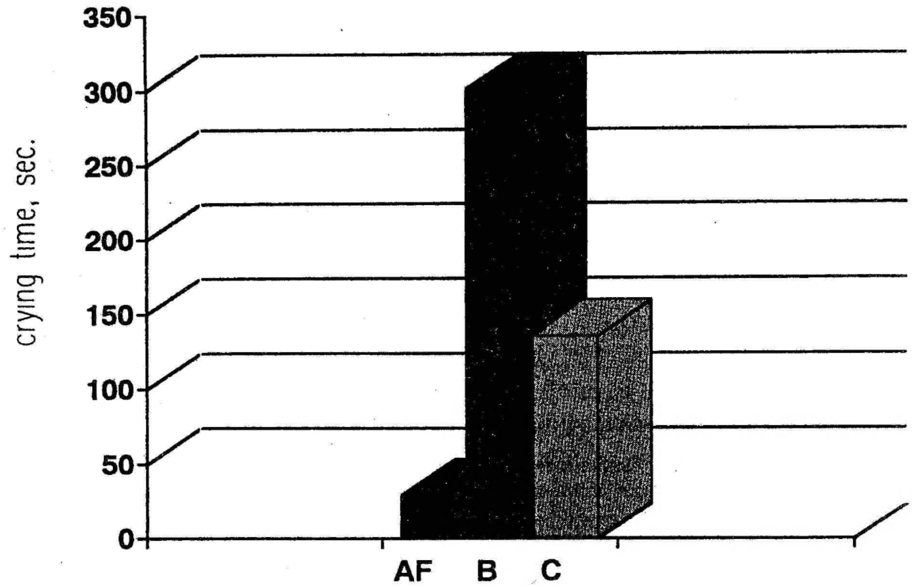

Fig. 2. Median crying time (s) during postnatal 31–90 min in babies assigned to amniotic fluid smell (AF—16 infants), breast odor (B—15 infants) and unexposed group used as controls (C—16 infants). Paired comparisons (Mann–Whitney U test) AF vs. B $p=0.02^*$ ($z=-2.4$); AF vs. C $p=0.02^*$ ($z=-2.3$); B vs. C $p=0.27$ ($z=-1.1$). Axis Y—crying time (s); axis X—AF, B, C odor exposure groups.

differences across the treatment conditions (see Table 2). Moreover, paired comparisons between conditions (Mann–Whitney U test) indicate that crying durations for infants exposed to AF odor were significantly lower than those of infants exposed to breast odors or controls (Fig. 2).

Median crying durations within each of the four 15-min blocks of time making up the 60-min observation period are summarised in Table 2. Kruskall–Wallis tests found significant differences between the three treatment conditions for each of the first three 15 min time blocks.

Mean axillary temperatures at postnatal age of 90 min did not differ reliably between groups, and remained >36.7°C in all three (Table 1). Likewise, no significant differences between treatment conditions were found for proportion of male/female infants, babies' birthweight, mothers' age and parity, or duration of the 1st or 2nd stage of labor.

4. Discussion

During the 60-min period beginning 30 min after birth, babies exposed to a cloth treated with AF spent less time crying than did neonates exposed to a pad previously worn over their mother's breast/axillae, or unexposed control babies. Since none of the subject infants had direct physical contact with the stimulus cloths or the

Table 2
Crying times in seconds (median and range) for the whole 60-min observation period and for each of the four 15-min periods in newborns exposed to odors of amniotic fluid, mother's breast and in non exposed controls

	AF odor ($n=16$)	Breast odor ($n=15$)	Control ($n=16$)	p-Value[a]
Total, min 31–90				
Median	29.3	301	135	0.02 (H=8.1)
(Range)	(0–167)	(0–1603)	(0–616)	
Min 31–45				
Median	0	39.8	1.3	0.03 (H=7.2)
(Range)	(0–51)	(0–380)	(0–194)	
Min 46–60				
Median	0	88.3	2.6	0.006 (H=10.1)
(Range)	(0–71)	(0–325)	(0–211)	
Min 61–75				
Median	2.9	51.6	34.6	0.03 (H=6.9)
(Range)	(0–36)	(0–534)	(0–242)	
Min 76–90				
Median	10.2	49.0	36.6	0.17 (H=3.5)
(Range)	(0–167)	(0–547)	(0–205)	

[a] Kruskall–Wallis test, comparison of three groups.

supporting funnel, the results most likely reflect olfactory differences across the three treatment conditions. Therefore, exposure to the odor of AF had a calming effect on newborn infants—at least as measured by their crying behavior. It can be assumed that in the natural setting the baby was held by the mother immediately after delivery without cleaning—as seen in the great apes—and was thus continuously exposed to the familiar odor of AF. This might have made the transition from intrauterine to extrauterine life appear less abrupt. The observed influence of AF odor on infants' crying is similar to the recently reported diminution of cries resulting from bodily contact with the mother [14]. In this latter study, however, it was not clear whether the suppression of crying was mediated by maternal auditory, tactile or olfactory cues (or a combination of these stimuli).

No reliable differences were observed in the crying durations for infants exposed to maternal breast odor vs. unexposed control infants. Nonetheless, for the total observation period the median crying time for babies in condition B was more than two times greater than that for the control babies. Moreover, the same pattern of results (B>C>AF) was consistently found across all four 15-min time blocks. This general trend for crying to increase among babies exposed to maternal breast odor is intriguing in light of our recent data indicating that olfactory cues emanating from the mother's breast help guide newborn infants to the nipple for their initial sucking bout [7]. Moreover, two-week-old infants, including those that had been exclusively formula-fed since birth, are attracted to breast odors produced by lactating females [6]. It therefore appears that maternal breast odors may signal the presence of an appropriate food source. Accordingly, if breast odors are present without allowing access to the nipple (as in the present experiment) infants may become disturbed or display heightened arousal. Macfarlane [17] had similarly noted that 2–10 day-old breastfed infants displayed increasing signs of frustration (heightened activity and crying) after their nose was in contact with a soiled breast pad for an "extended time" (during the 60-s test period).

In general, the duration of crying by the control babies in the present experiment was considerably less than that observed in a group of neonates of the same age tested in Madrid [14]. A possible explanation for this discrepancy may be the differential postnatal care of the newborns; e.g., unlike the babies in Spain, those in Estonia were swaddled and placed under a radiant heater, and also had a slightly higher mean axillary temperature.

Reduced crying among babies exposed to the odor of AF is consistent with the hypothesis that the fetus may become familiar with chemical cues present in the intrauterine environment. During the final trimester of gestation, odorous substances in the AF may come into contact with nasal chemoreceptors as it is inhaled by fetus. The newborn infant might retain a memory trace of such prenatal olfactory experience and therefore respond discriminatively to those same cues during the early postnatal period [18]. This hypothesis is further supported by experiments with rats (mentioned in Section 1) documenting that the manner in which newborn pups respond to specific odors varies as a function of prior prenatal experience with those scents [10,11].

Our data provide new evidence of the human baby's fine olfactory discrimination capacity, and add to the growing body of evidence indicating that naturally occurring odors play an important role in the mediation of infants' early behavior. In addition to AF, odors emanating from the breasts of lactating women are also attractive to neonates [6] and help guide them to the nipple [7]. Changes in the mother's diet alter the odor/flavor of her milk which in turn influences the baby's sucking behavior [19]. Because mother's diet also flavors her AF [20,21], the odor and gustatory profiles of her AF and milk may be somewhat similar [10,22]. Thus, familiarization with the prenatal chemical environment may facilitate the baby's acceptance of mother's milk when first put to the breast. Moreover, within several days after birth, breast-fed infants are capable of recognising their mother by the unique olfactory signature associated with her breasts and axillary region [17,23].

5. Notation

AF—amniotic fluid

Acknowledgements

The authors acknowledge the financial support by the Karolinska Institute and the Foundation "Frimurare Barnhuset".

References

[1] Chuah MI, Farbman AI. Developmental anatomy of the olfactory system. In: Doty RL, editor. Handbook of olfaction and gustation. New York: Marcel Decker, 1995:147–170.
[2] Leon M, Moltz H. The development of the pheromonal bond in the albino rat. Physiol Behav 1972;8:683–6.
[3] Porter RH, Doane HM. Maternal pheromone in the spiny mouse (*Acomys cahirinus*). Physiol Behav 1976;16:75–8.
[4] Blass EM, Teicher MH. Suckling. Science 1980;210:15–22.
[5] Hudson R, Distel H. Nipple location by newborn rabbits: behavioral evidence for pheromonal guidance. Behaviour 1983;85:260–75.
[6] Porter RH, Makin JW, Davis LB, Christensen KM. An assessment of the salient olfactory environment of formula-fed infants. Physiol Behav 1991;50:907–11.
[7] Varendi H, Porter RH, Winberg J. Does the newborn baby find the nipple by smell? Lancet 1994;344:989–90.
[8] Schaal B, Marlier L, Soussignan R. Responsiveness to the odour of amniotic fluid in the human neonate. Biol Neonate 1995;67:397–406.
[9] Varendi H, Porter RH, Winberg J. Attractiveness of amniotic fluid odor: evidence of prenatal learning?. Acta Paediatr 1996;85:1223–7.
[10] Hepper PG. Adaptive fetal learning: prenatal exposure to garlic affects postnatal preferences. Anim Behav 1988;36:935–6.
[11] Smotherman WP. Odor aversion learning by the rat fetus. Physiol Behav 1982;29:769–71.
[12] Schapiro S, Salas M. Behavioral response of infant rats to maternal odor. Physiol Behav 1970;5:815–7.
[13] Oswalt GL, Meier GW. Olfactory, thermal and tactual influences on infantile vocalizations in rats. Dev Phychobiol 1975;8:129–35.
[14] Christensson K, Cabrera T, Christensson E, Uvnäs-Moberg K, Winberg J. Separation distress call in the human neonate in the absence of maternal body contact. Acta Paediatr 1995;84:468–73.
[15] Michelsson K, Christensson K, Rothgänger H, Winberg J. Crying in separated and non-separated newborns: sound spectrographic analysis. Acta Paediatr 1996;85:471–5.
[16] Sullivan R. Attenuation of crying in newborns by maternal odors. XIVth Biennial Meeting of the International Society for the Study of Behavioral Development (Quebec), 1996:Abstract.
[17] Macfarlane A. Olfaction in the development of social preferences in the human neonate. In: Porter R, O'Connor M, editors. Parent–infant interaction (Ciba Found. Symp. 33), New York: Elsevier, 1975:103–113.
[18] Hepper PG. Human fetal "olfactory" learning. Int J Prenatal Perinatal Psychol Med 1995;7:147–51.
[19] Mennella JA, Beauchamp GK. The transfer of alcohol to human milk—Effects on flavor and the infant's behavior. New Engl J Med 1991;325:981–5.
[20] Mennella JA, Johnson A, Beauchamp GK. Garlic ingestion by pregnant women alters the odor of amniotic fluid. Chem Senses 1995;20:207–9.
[21] Hauser GJ, Chitayat D, Berns L, Braver D, Muhlbauer B. Peculiar odours in newborns and maternal prenatal ingestion of spicy foods. Eur J Pediatr 1985;144:403.
[22] Stafford M, Horning MG, Zlatkis A. Profiles of volatile metabolites in body fluids. J Chromatogr 1976;126:495–502.
[23] Cernoch JM, Porter RH. Recognition of maternal axillary odors by infants. Child Dev 1985;56:1593–8.

V

Varendi H., Porter R. H.
Maternal breast odor influences newborn's
spontaneous prefeeding behavior. Infant Behavior and Development
(submitted for publication).

MATERNAL BREAST ODOR INFLUENCES NEWBORN'S SPONTANEOUS PRE-FEEDING BEHAVIOR

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Abstract

In previous studies, newborn infants placed on their mother's chest displayed a sequence of pre-feeding behaviors, grasped a nipple and sucked without assistance. Furthermore, neonates sucked preferentially from an untreated breast rather than the alternative breast that had been washed to eliminate its natural odor. We have further investigated the influence of breast odors on the sequence of behavior that typically precedes spontaneous breastfeeding. Twenty-two babies were observed during two trials on a warming bed. In one trial, a soiled breast pad was placed 17-cm in front of the baby's nose; in the other trial a clean pad was used. More babies moved towards and reached the breast pad than the clean pad. Latencies to lift the head, push with the legs, and move forward were significantly shorter during the breast pad trials. Natural breast odors accelerate pre-feeding behaviors and are sufficient to guide neonates to the odor source.

Key words: breast odor, newborn, olfaction, pre-feeding behavior

INTRODUCTION

When newborn infants are placed on the mother's chest immediately after birth, or within several days postpartum, they spontaneously crawl to the nipple and begin to feed (Widström *et al.*, 1987; Varendi *et al.*, 1996, 1997). Grasping the nipple and sucking are preceded by a stereotyped pattern of pre-feeding behavior including: rooting, head turning, hand-mouth contact and crawling leg movements. In several experiments, babies oriented preferentially to their mother's unwashed breast rather than the alternative breast that had been thoroughly washed and rinsed prior to the test trial (Varendi *et al.*, 1994; 1997). It therefore appears that natural odors emanating from the nipple/areola help guide the neonate to the nipple. However, these earlier studies did not assess whether sensory input other than olfactory stimuli contribute to the complex chain of behaviors that end in a sucking bout. That is, the mother is the source of an array of visual (Bushnell *et al.*, 1989), auditory (Fifer & Moon, 1994) and tactile (Christenssson *et al.*, 1995; Uvnäs-Moberg, 1996) stimulation that the infant may perceive and respond to when placed on her bare chest. In an attempt to elucidate further the role of olfaction per se in early pre-feeding behavior and orientation to the breast, we observed babies when additional maternal cues (e.g. voice, skin temperature and texture, body form, heart and respiratory sounds and movement) were not available.

METHOD

Subjects

Twenty-two healthy term newborns, 10 boys and 12 girls were recruited for the study within the first hours after birth. Their mothers were asked to participate and keep a breastfeeding diary during their stay in the maternity ward. Oral informed consent was obtained from the mothers and the study was approved by the Ethics Committee at Tartu University.

Testing procedure

On the morning of the test day (postnatal day 2–4), the mother was asked to fasten a round cotton pad (diameter ~4 cm) above the nipple on each of her breasts so that the lower edge of the pad touched the nipple but did not cover it. Pads were fixed with silk tape after the routine morning visit of the pediatrician and following the morning breastfeeding. Mothers were asked to keep these pads on the breasts for at least 2 hrs. Testing was performed when the baby had spontaneously woken and was presumably hungry (preferably >2 hrs since the previous breastfeeding, see Table 1). Each baby was tested two times; once with a soiled breast pad (B) and once with a clean control pad (C), in a randomized order.

The tests were performed in the nursery with room temperature 22–24°C. A warming bed (Babytherm 4200, Dräger, Germany) covered with cotton linen and set at 37°C, with a surface temperature of 29±0,5°C, was used. Three silk tapes scaled by 1 cm (0–21 cm) were fixed on the linen covering the mattress, see Figure 1.

Before the first test session began, the baby was undressed. The mother took off her breast pads (only one of these pads was used for test purposes) and assisted with the test. She was asked to use the stimulus pads in the sequence given in her written instructions. She placed pad 1 (breast pad or clean pad) on a piece of plastic foil (diameter ~5 cm) that was in turn positioned on the mattress of the warming bed so that the lower and upper margins of the pad were between the 17 and 21 cm marks respectively on the central tape scale. The observer was not aware of which pad (B or C) was used in a given test session.

After the pad was in place, the observer placed the naked baby prone on the warming bed with the tip of the nose on the midline, touching the 0–point of the central tape scale (start position, Figure 1). At that moment, the stopwatch was switched on and the baby's behavior (see below) recorded according to the study protocol. Observations lasted a maximum of 180 sec. The test was terminated if the baby reached the pad (forehead touched the pad and moved it from its original position) before 180 sec. had elapsed. After the first test, the baby and the test pad (along with the foil) were removed from the warming bed. Following a 2-min pause, the mother placed test pad 2 with its foil backing onto the warming bed and the second test trial was conducted as described for test 1.

Testing order was randomised as follows:

Order 1 (11 babies): test 1 — C; test 2 — B;

Order 2 (11 babies): test 1 — B; test 2 — C.

The main characteristics of the babies in these two testing orders and their relevant pretest experience are given in Table 1. There were no statistically reliable differences between the babies in the two test orders for any of the variable.

Outcome measures

The location of the baby's nose (distance from the start position, cm) was recorded at 15, 30, 45, 60, 90, 120, 150 and 180 seconds after the observations began.

At these same time points, the observer noted the baby's state of arousal (e.g. calm; crying; bodily movements).

In addition, the following behavioral measures were recorded:

- latency of initial head turn to one side (the first head turn after initial placement of the baby, both eyes turned to one side of the midline, cheek on the surface of the mattress)
- number of additional head turns
- latency until the first hand-mouth contact (contact of fist or fingers with lips, >2 sec)
- duration of sucking of fingers or wrist
- latency until the start of rooting (when the baby started to search in the midline)
- latency until the first lift of the head
- latency to pushing with legs
- latency to move towards the stimulus pad (nose moves forward more than 2 cm during the interval between two successive observation points).

RESULTS

The most noticeable differences in behavior between the two test conditions, i.e. breast pad and control pad (B/C), concerned movement from the start position. Overall, 21/22 babies moved towards B, while only 7/ 22 babies moved in the direction of C (Table 2). All 7 babies who moved towards the control pad did so during the second test trial and had previously moved toward the breast pad during test trial 1. Furthermore, 18 babies advanced to touch the breast pad with their forehead (nose distance at that time >13 cm from the start position), as compared to 3 babies who reached the control pad (McNemar's test, $z=3.87$, $p<.0005$). The latter 3 babies reached the control pad during test trial 2 and had all reached the breast pad during the preceding test (trial 1).

For all additional measures, the test order, i.e. C followed by B, or vice versa — orders 1 and 2 — did not significantly influence the results. Therefore, the data from the 2 testing orders were collapsed and the results are presented as responses to breast pads (B; n=22) and control pads (C; n=22) (Table 2).

As indicated in Table 2, most babies (while awake and presumably hungry) displayed features of the typical pre-feeding behavioral sequence when exposed to either the breast or control pad. Infants tested with B in comparison to C had significantly shorter latencies for each of the following measures: initial lifting of the head, pushing with legs and movement towards the stimulus pad. As observed previously in tests performed 2–4 days postpartum (Varendi *et al.*, 1997), only a minority of the babies showed hand-mouth contact and finger sucking during the observation period. During many of the test trials (23/44), the infants displayed angry continuous crying at >50% of the observation points, while actively rooting and pushing.

The differences in responses to the two pads can be further quantified by using the centimetre scale to determine nose distance from the starting point (in the direction of the stimulus pad). Sixty-sec. after the test session began, and at all subsequent observation times, the median distance advanced by babies tested with a breast pad was significantly greater than the distance moved by babies exposed to a clean control pad (Figure 2).

DISCUSSION

Comparisons between the behavior observed in conditions B vs. C indicate that forward movement in the direction of the stimulus pad was greatly increased (and with a shorter latency) in response to maternal breast odors. Significantly more babies reached the breast pad than the control pad, and the median distance covered during the 180-sec. test session was greater in Condition B. We cannot judge whether this was due to stronger pushing with the legs or to a better coordination of movements, when the babies were stimulated by the breast odors. (It is known that exposure to a human voice modifies the jerky arm movements of newborns and makes them more smooth and seemingly more "coordinated"). Infants also displayed reduced latencies for head lifting and pushing with their legs during the breast-pad tests. It therefore appears that breast odors — in the absence of additional maternal cues — accelerate the sequence of pre-feeding behaviors and are sufficient to guide the baby to the odor source. This physical attraction to maternal breast odor corroborates the results of earlier related studies showing that babies preferentially turned their head in the direction of a pad bearing the odor of their mother's breast (Macfarlane, 1975). Moreover, bottle-fed infants oriented preferentially towards the odor of a breast pad from an unfamiliar lactating female, even when such cues were simultaneously paired against the scent of the infants' familiar formula (Makin & Porter, 1989; Porter *et al.*, 1991). Odors emanating from the breasts of nursing mothers therefore appear to function as general attractants for neonates.

In a more natural context, when babies were placed on their mother's chest within minutes after birth, a significant majority spontaneously chose to suck from the unwashed (naturally smelling) breast rather than the alternative breast that had been thoroughly washed to remove any olfactory cues (Varendi *et al.*, 1994). Grasping the nipple and sucking was preceded by a stereotyped sequence of behavior similar to that described above (including pushing with the legs and crawling movements). The results of the present study are further evidence that odors emanating from the mother's breasts contribute to nipple localization and effective breastfeeding in this natural situation. Olfactory stimuli therefore appear to have a greater importance in breastfeeding behavior and physiology than is generally believed.

Babies' responses in condition C varied to some extent as a function of the testing order. All of the 7 babies who crawled towards the control pad had previously been tested with the breast pad and had also reached it (Order B/C). Thus, no babies reached pad C when tested with that pad during test trial 1 (Order C/B). One possible explanation for this effect of prior testing with maternal breast odor may be that the motor activity elicited by that scent during the first test trial persisted through test session 2 with the control pad. Alternatively, babies may have learned to associate forward movement with reaching the breast pad in trial 1, and therefore repeated that behavior in trial 2 — even though the breast odor was no longer present. In any event, these data illustrate the complex influence of olfactory cues on neonatal behavior.

Several categories of pre-feeding behavior (rooting, head turning, hand-mouth contact) were similar in conditions B and C, suggesting that they may have been elicited by the prone position and hunger rather than odors or other external stimuli. However, there were marked differences in head turning by the babies in the present study when compared to the pre-feeding behavior shown by babies in an earlier experiment (Varendi *et al.*, 1997) who were tested for spontaneous nipple localization when placed on their mother's chest. When allowed to choose between their mother's untreated breast and the alternative breast (either washed or treated with a small amount of amniotic fluid), 40 of 57 babies displayed 2 or more lateral head turns — i.e. towards each of the breasts — before grasping the nipple. In the present study, however, where there was either no olfactory stimulus (condition C), or the breast odor was presented in front of the baby (condition B), more than one lateral head turn was recorded in only 9 of the 44 trials. The increased number of lateral head movements in response to olfactory differences between the mother's breasts most likely reflects infants' active sampling of those two odor sources. When the salient breast odors are positioned in front of the infant, however, lateral head movements would not facilitate perception or localization of the olfactory cues.

The role of maternal breast odors in eliciting goal directed behavior in human infants is functionally similar to that of "nipple search pheromone" for newborn rabbits (Distel & Hudson, 1985), rat pups (Blass & Teicher, 1980) and piglets (Morrow-Tesch & McGlone, 1990). Neonates of each of these animal species are dependent upon odorous signals from the mother's ventrum to locate the nipples. Elimination of these chemical attractants, or interfering with neonates' olfactory perception, severely disrupts successful nipple attachment and sucking. Newborn humans likewise appear to be sensitive to alterations in their mother's naturally occurring breast odors (Varendi *et al.*, 1994; 1997; Mennella & Beauchamp, 1991).

ACKNOWLEDGEMENTS

Our sincere gratitude to Jan Winberg for his valuable guidance and discussions.

The study was supported by grants from the Foundation "Frimurare Barnhuset" and Tartu University (ARLA 0475).

REFERENCES

Blass, E. M. & Teicher, M. H. (1980). Suckling. *Science, 210*, 15–22.

Bushnell, I. W. R., Sai, F. & Mullin, J. T. (1989). Neonatal recognition of the mother's face. *British Journal of Developmental Psychology*, 7, 3–15.

Christensson, K., Cabrera, T., Christensson, E., Uvnäs-Moberg, K. & Winberg, J. (1995). Separation distress call in the human neonate in the absence of maternal body contact. *Acta Paediatrica, 84*, 468–473.

Distel, H. & Hudson, R. (1985). The contribution of the olfactory and tactile modalities to the nipple search behaviour of newborn rabbits. *Journal of Comparative & Physiological Psychology*, A, 157, 599–605.
Fifer, W. P. & Moon, C. M. (1994). The role of mother's voice in the organiza-tion of brain function in the newborn. *Acta Paediatrica*, Suppl. 397, 86–93.
Macfarlane, A. (1975). Olfaction in the development of social preferences in the human neonate. In R. Porter & M. O'Connor (Eds.), *Parent-Infant Interaction* (Ciba Foundation Symposion, 33) (pp. 103–113). New York: Elsevier.
Makin, J. W. & Porter, R. H. (1989). Attractiveness of lactating females' breast odors to neonates. *Child Development, 60,* 803–810.
Mennella, J. A. & Beauchamp, G. K. (1991). Maternal diet alters the sensory qualities of human milk and the nursling's behavior. *Pediatrics, 88,* 737–744.
Morrow-Tesch, J. & McGlone, J. J. (1990). Sensory systems and nipple attachment behavior in neonatal pigs. *Physiology & Behavior, 47,* 1–4.
Porter, R. H., Makin, J. W., Davis, L. B. & Christensen, K. M. (1991). An assessment of the salient olfactory environment of formula-fed infants. *Physiology & Behavior, 50,* 907–911.
Uvnäs-Moberg, K. (1996). Neuroendocrinology of the mother-child interaction. *Trends Endocrinol. Metab.*, 7, 126–131.
Varendi, H., Porter, R. H. & Winberg, J. (1994). Does the newborn baby find the nipple by smell? *Lancet, 344,* 989–990.
Varendi, H., Porter, R. H. & Winberg, J. (1996). Attractiveness of amniotic fluid odor: evidence of prenatal learning? *Acta Paediatrica, 85,* 1223–1227.
Varendi, H., Porter, R. H. & Winberg, J. (1997). Natural odour preferences of newborn infants change over time. *Acta Paediatrica, 86,* 985–990.
Widström, A. M., Ransjö-Arvidson, A. B., Christensson, K., Matthiesen, A.-S., Winberg, J. & Uvnäs-Moberg, K. (1987). Gastric suction in healthy newborn infants. *Acta Paediatrica Scandinavica, 76,* 566–572.

Table 1. Characteristics of the Test Order Groups 1 and 2

	Group 1 (C / B)	Group 2 (B / C)
Boys / Girls	5 / 6	5 / 6
Birth weight, g	3740 (2950–4540)	3510 (3130–4160)
Age at the observation, hrs	60 (49–80)	55 (36–71)
Number of breastfeedings preceding the test	16 (8–26)	17 (6–24)
Cumulative breastfeeding time, min.	280 (85–365)	285 (165–630)
Duration of mother-infant close contact, hrs	39 (17–53)	26 (19–52)
Interval between the last breastfeeding and testing, hrs	2,5 (2–3,5)	2,5 (1,8–5)
Length of time the breast pad was worn by the mother, hrs	2,3 (2–3)	2,5 (1,9–4)

Note. Data given as medians (range).

Table 2. Medians (range) of Pre-Feeding Behavioral Measures in Response to Breast Odor Pad (B) or Control Pad (C).

	Exposure to B (n = 22)		Exposure to C (n = 22)	
Start of rooting at	8 (1–180)	n = 19	13 (1–180)	n = 17
Initial turn of head at	18 (1–180)	n = 14	13 (5–180)	n = 18
Number of additional head turns	0 (0–2)	n = 4	0 (0–2)	n = 5
First lift of head at	15 (1–180)	n = 18	63* (5–180)	n = 15
Hand-mouth contact at	180 (5–180)	n = 8	165 (15–180)	n = 11
Duration of finger sucking	0 (0–20)	n = 5	0 (0–100)	n = 8
Start of pushing with legs at	5 (1–180)	n = 21	8* (1–180)	n = 19
Start of movement at	50 (5–180)	n = 21	180** (5–180)	n = 7

Note. Latency and duration measures given in sec.; n = no. of babies showing this behavioral characteristic. * $p<0.05$; ** $p<0.01$.

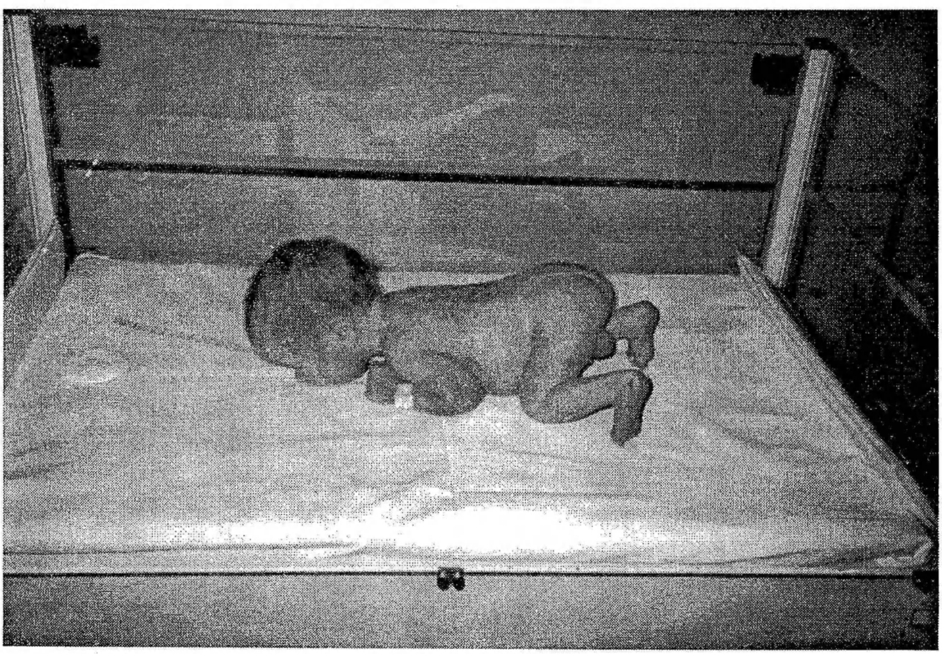

Figure 1. Baby on the warming bed in the start position, cm scales and pad on foil.

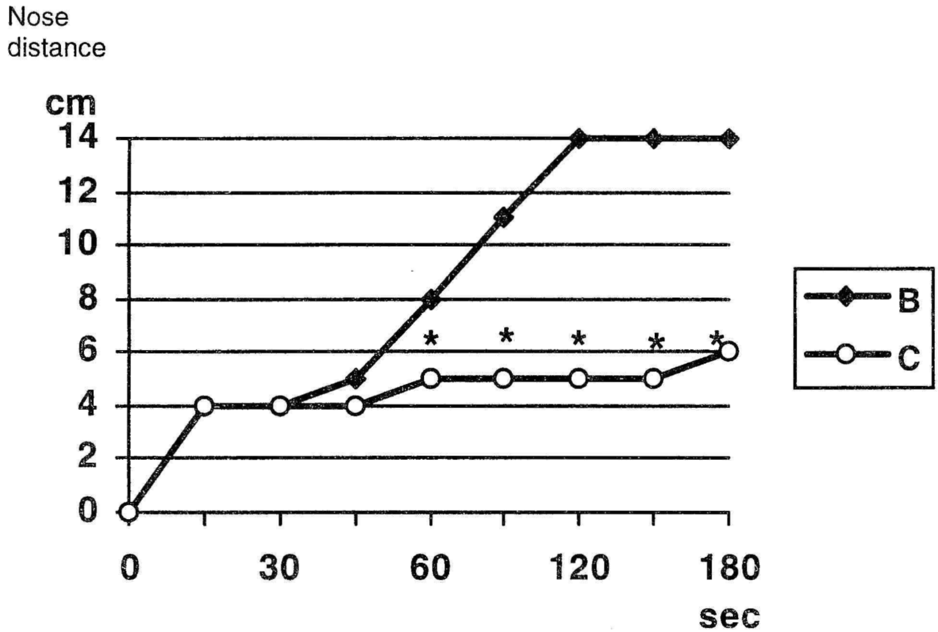

Figure 2. Median nose distances (cm) from the start position for babies during the breast pad (B) and control pad (C) tests; time points given in sec. since the start of observation.

* $p<0.01$ [Note that the nose was at 14 cm when the forehead touched the pad.].

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Teadustöö

Põhiliseks uurimisvaldkonnaks on olnud vastsündinu käitumise ja haistmise seosed. Uurinud ka toitmise mõju vastsündinu soole mikrofloora kujunemisele ning analüüsinud Eesti perinataalmeditsiini probleeme.

12 teaduspublikatsiooni, kaheksa ettekannet rahvusvahelistel konverentsidel.

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ISSN 1024–395X
ISBN 9985–56–429–4