

The effect of the mothers' smell on the sleeping behavior of infants in daycare

Name: KS Illingworth
Student number: S0729604
Specialization: Child and Family Studies, University of Leiden
Supervisors: Rodrigo Cárcamo M.Sc. and dr. Lenneke Alink
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Preface

This thesis is part of the ‘smell study’ of the University of Leiden. This study investigated the effect of the smell of the own mother compared to the smell of another mother and a neutral smell on the sleeping- and crying behavior of infants in daycare. I was immediately interested to participate in this study, because it is an interesting subject and it provided me with the opportunity to be part of the research from the beginning to the end. A lot of studies that were available at the time, were larger studies where I could only participate in a specific part of the whole research project. The current study was a good opportunity to experience research for the beginning to the end, because now I got acquainted with all the aspects of research and not just one part. Together with fellow student Tessa Zitman, we were responsible for the organization and execution of the study. We were a very good team, and despite all the stressful phone calls to each other, everything turned out just fine. Rebeca Sandu, Lisa Orlandini and Moheb Elwakiel helped with coding all the video’s and did some visits as well.

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ABSTRACT: In this study the effect of the smell of the own mother, the smell of another mother and a neutral smell on the sleeping behavior of infants (n=23) in daycare was investigated. Infants (<36 months) who attend daycare have higher stress levels, and for the regulation of their stress, sleep is very important. Previous research showed that the smell of the mother can have a soothing effect on the child during pain; children stopped crying sooner when they smelled their own mother, but is there also an effect when infants are falling asleep in daycare? Does temperament of the child have an influence on this effect? Results showed no significant effect of type of smell on the sleeping behavior of the child. In addition, no significant effects were found of temperament on the effect of type of smell on sleeping behavior. The lack of significant results can be explained by the fact that the infants in this study were young and not used to daycare yet, and they did not have a real difficult temperament. Further research with a larger sample and older children, who are attending daycare at least twice a week and for a longer period of time, is needed, because the smell of the own mother may work as an intervention.

Introduction

Attending daycare has an influence on the stress regulation of young children (Vermeer & Van IJzendoorn, 2006). Morning naps are significantly associated with a decrease in cortisol, the stress hormone (Larson, Gunnar & Hertzgaard, 1991). Younger children have more secretion of cortisol in daycare compared to older children (Vermeer & Van IJzendoorn, 2006). It is important for the regulation of their cortisol level to have sufficient sleep in daycare, especially for young children (<36 months). What if children have difficulty sleeping in daycare? The familiar smell of their own mother can have a soothing effect on the child during pain (Goubet, Rattaz, Pierrat, Bullinger & Lequien, 2003; Rattaz, Goubet & Bullinger, 2005). Placing an object with the smell of their own mother in the crib of the infant in daycare may be a solution for inducing sufficient sleep in daycare. This study investigates if children (3-6 months) who attend daycare sleep better (defined as short time falling asleep, long total duration of sleep, little wake minutes and a good quality of sleep) if they smell their own mother compared to the smell of another mother or a neutral smell.

Several researchers concluded that young children, who attend daycare, have more stress during the day compared to when they stay home (Dettling, Gunnar & Donzella, 1999; Vermeer & Van IJzendoorn, 2006). Several studies were conducted to investigate the effect of going to daycare on the stress regulation of the child. In the study by Dettling and colleague's (1999) cortisol was collected during the day from children (median = 49 months, range 39-69 months) who go to daycare. These children had an increase instead of a decrease in cortisol during the day. The same result was found in a meta-analysis: children who go to daycare have higher cortisol levels during the day compared to when they stay at home and they also have an increase instead of a decrease in cortisol during the day. (Vermeer & Van IJzendoorn, 2006).

Sleep is very important for the regulation of cortisol in the home setting (Larson et al., 1991). Morning naps at home are associated with a significant decrease in cortisol. A lower level of cortisol was measured in infants (M=9.4 months) who just woke up after a morning nap than before they went to sleep. Two-year-olds (M=24.5 months) who woke up more frequently during sleep at home had higher cortisol levels than children who woke up less frequently (Scher, Hall, Zaidman-Zait & Weinberg, 2009). Contradictory, in older children the increase in cortisol in daycare centers is not significantly related to napping variables (Watamura, Sebanc & Gunnar, 2002), other factors than daytime rest periods seem to play a role in the increase in cortisol of older children (M= 3.45 years) who attend daycare.

However, cortisol was significantly higher on days that the child napped than on days that the child did not nap. These inconsistent results can be the result of age differences between the studies. The effect of daycare on the secretion of cortisol is most apparent in children younger than 36 months (Vermeer & Van IJzendoorn, 2006). Several studies with young children found an effect of sleep on cortisol (Larson et al., 1991; Scher et al., 2009), but this is not found in studies with older children (Watamura et al., 2002). Age is an important factor to take into account when considering the effect of sleep on cortisol. Sufficient morning naps in daycare may only regulate young children's cortisol levels. Another factor that seems to influence cortisol is temperament. There is some evidence that children with a difficult temperament exhibit less of a decrease in cortisol after napping than children with an easy temperament (Watamura et al., 2002). A good sleep pattern also has an influence on cortisol, which is important for the adjustment in preschool children (Bates, Viken, Alexander, Beyers & Stockton, 2002).

Going to daycare is a stressful event for many infants, because of the prolonged separation from the parent(s), multiple and frequent changes of caregivers and interacting with peers in a social group. It is important that babies, especially those with a difficult temperament, have sufficient sleep in the daycare centre, so their stress can be regulated and they can adjust better later in life.

The preference for smell

Newborns can develop a preference for a certain smell almost immediately after birth (Balogh & Porter, 1986; Varendi, Porter & Winberg, 1994). When healthy baby girls were exposed to an artificial smell within the first days of life, they develop a preference for this smell (Balogh & Porter, 1986). This was not found in boys. Varendi and colleague's (1994) found that newborns can find the nipple by the smell, without having any experience of breastfeeding. When babies have experienced breastfeeding they develop a preference for the smell of their own mothers' breast compared to the smell of another nursing mother (Macfarlane, 1975, as described in Porter & Winberg, 1999). In this study mothers wore a gauze pad in their bra and this was placed near a six-day old baby. The newborns orientated their head more to the pad worn by their own mother than to the pad worn by another nursing mother. Newborn babies develop a preference for the smell of the breast of their own mother almost immediately after birth. The same result is found in older babies (Cernoch & Porter, 1985; Russell, 1976). When a breast pad worn by their own mother was presented to the six-

week old breastfed baby, they moved their head more toward this pad and displayed more sucking movement compared to a breast pad worn by a strange nursing woman and a pad treated with cows' milk (Russell, 1976).

This orientation towards the breast of lactating mothers is not consistently found when babies are formula-fed. In the study of Cernoch and Porter (1985) mothers wore a gauze pad under their arm for approximately eight hours. This pad was then placed near the child and their head orientation was monitored. Results showed that bottle-fed infants cannot discriminate between the smell of their own mother, the smell of another non-lactating mother and another lactating mother. However, 2-week-old formula-fed babies orientated more towards the breast pad with the smell of their own mother compared to a clean control pad (Porter, Makin, Davis & Christensen, 1991). When both the smell of the breast of their own mother and the smell of formula milk were presented to the baby, the preference for the breast was no longer apparent. They orientated their head more towards the smell of formula milk than to the smell of their own mother. However, a difference between boys and girls is found. Baby boys preferred the smell of formula milk to the smell of the breast of their own mother, while girls did not have a preference for either smell.

In addition, another study demonstrated that formula-fed girls, who did not have experience with breastfeeding, did have a preference for the smell of mother milk (Makin & Porter, 1989). They even preferred the smell of mother milk from another mother to the smell of formula milk. Marlier and Schaal (2005) investigated whether infants had a preference for human milk or formula milk. As noted above, breastfed infants develop a preference for the smell of their own mother, but results are inconsistent for formula-fed infants. When the smell was unfamiliar both breastfed and formula-fed babies orientated their head more towards the smell of human milk. The same result was found for mouthing; despite feeding experiences both groups mouthed more when they smelled mother milk. Even when the baby was presented with *unfamiliar* human milk and *familiar* formula milk, they mouthed more when they smelled human milk. Results showed that despite feeding experience, babies orientated more to the breast of lactating mothers (Porter & Winberg, 1999).

Some researchers argue that there is a sensitive period for the development of a preference for a certain smell (Romantshik, Porter, Tillman & Varendi, 2006). Babies who were exposed to a smell between 4-37 minutes after birth developed a preference for this smell, but when the babies were exposed to this smell after twelve hours postnatal they did not develop this preference. Russell (1976) investigated if newborns can discriminate between

the smell of their own mother, the smell of another mother and no smell. Mothers wore a cotton pad in their bra for three hours. Then the pad was placed in the crib of a sleeping baby at three time points: two days, two weeks and six weeks after birth. Only at the second and third time point the babies responded to the different smells. Two weeks after birth eight out of ten babies responded to the smell of the other mother and one out of ten babies responded to the smell of the own mother. Six weeks after birth there was a different picture: only one out of ten responded to the smell of the other mother, while seven out of ten responded to the smell of the own mother. Sleeping newborns cannot discriminate between the smell of the own mother and the smell of the other mother up to six weeks postnatal. The same study was done by Sullivan and Toubas (1998). They investigated if the smell of the own mother had an effect on the crying, sleeping and awake newborn compared to the smell of another mother, a neutral smell or no smell. There was no difference between all four smells noted for sleep, but in this study the newborns were already asleep. Maybe there will be an effect when the time before the infant falls asleep is measured. This will be tested in the current study. Nevertheless, for awake newborns there are different results than when the babies were already asleep, because they could discriminate between the smell of their own mother compared to the other three smells (Sullivan & Toubas, 1998).

The influence of smell

A smell can have a soothing influence on babies (Nishitani, Miyamura, Tagawa, Sumi, Takase, Doi et al., 2009; Rattaz, Goubet & Bullinger, 2005). A method that is frequently used in nurseries is to place an object with the smell of the own mother in the crib of the infant (Sullivan & Toubas, 1998). It was investigated whether the smell of the own mother had a positive influence on the crying-, sleeping- or awake infant compared to the smell of another mother, a neutral smell or no smell. They tested this with hospital gowns worn by mothers who just gave birth. Babies who were breastfed and babies who were formula-fed sooner stopped crying when they smelled a mother. There was no difference between the smell of their own mother and the smell of another mother.

Smell can also have an influence during pain (Goubet, Rattaz, Pierrat, Bullinger & Lequien, 2003; Rattaz et al., 2005). Goubet and colleague's (2003) investigated if babies cried less during the heel stick if they smelled a familiar smell, an unfamiliar smell or no smell. Babies who smelled an unfamiliar smell or no smell cried more during the heel stick than babies who smelled a familiar smell. The same result was found in the research of Rattaz and

colleague's (2005). One group of the newborn babies who participated in this study were familiarized with the smell of mother milk or the smell of vanilla. They constructed four groups in total; the mother milk group, the familiar vanilla group, the unfamiliar vanilla group and the control group. Babies who smelled a familiar smell (mother milk or vanilla) cried less, grimaced less and had less head movement during the heel stick. Especially the familiarity with a certain smell can have a soothing effect during the heel stick. This was also found in the study of Nishitani and colleague's (2009). When babies smelled the milk of their own mother during the heel stick, they cried less, grimaced less and moved less than babies who smelled the milk of another mother, the smell of formula milk or were not presented with a smell. The mother milk of their own mother has a soothing effect on infants during pain.

Skin-to-skin contact is important for recognizing the smell of the mother (Mizuno, Mizuno, Shinohara & Noda, 2004). Newborn babies were distributed in two groups: one group had immediate skin-to-skin contact with the mother after birth and the other group did not have immediate physical contact with the mother after birth. Newborns in the first group recognized the smell of the mother milk quicker than the group who had no skin-to-skin contact within 50 minutes after birth. When the effect of a certain smell on the drinking behavior of newborn babies is investigated the following can be concluded. When premature babies smelled mother milk before they would get breastfeeding, they had longer sucking reflexes and drank significantly more than premature babies in the control group (Raimnault, Saliba & Porter, 2006). Also in a group of college students the effect of smell during sleep is apparent. (MacBurney, Shoup & Streeter, 2006). Both men and woman during a separation of their partner were happier, more comfortable and felt safer when they smelled the smell of their partner during sleep.

Temperament and sleep

Temperament can be conceptualized in many different ways (Goldsmith, Buss, Plomin, Rothbart, Thomas, Chess, et al., 1987). The core concept of temperament is that there are individual differences in activity and regulation (Rothbart, Ahadi & Evans, 2000). Both genetic and non-shared environment play a role in the expression of temperament (Saudino, 2005).

One child sleeps better than the other child. Is this a consequence of differences in temperament? In a study by Weissbluth and Liu (1983) sleeping behavior of babies was investigated through parental reports. Babies with a difficult temperament slept for shorter

periods than babies with an easy temperament. Boys were more often described as more difficult than girls. The same can be concluded for the frequency of waking at night (Carey, 1974). It was investigated if certain child characteristics influence the amount of night wakening. A baby was defined as having a sleep problem if the baby woke up and cried more than once between midnight and 5 AM, and if this happened at least four out of seven nights during the first six to twelve months of their lives. Results show that a low sensory threshold, measured through a temperament questionnaire, correlated significantly with the amount of night wakening. When behavioral measures of temperament are used it appears that children with a difficult temperament sleep less and wake up more frequently during the night than children defined as 'easy' (Halpern, Anders, Coll & Hua, 1998).

Conclusive results about the role of temperament on sleep are not always found. The study by Scher, Tirsoh and Lavie (1998) with infants (M=12 months) found that there is only a weak relation between temperament and the sleep pattern. The authors argue that the weak association was the result of the use of a maternal perception questionnaire of infant temperament instead of more informants or an objective measurement of infant temperament. In adults there was also a weak association between temperament and total sleep time (Ottoni, Lorenzi & Lara, 2010). A high score on anger was associated with a higher amount of night wakening.

Sometimes differences in perception of infant temperament between mothers and fathers are found (Keener, Zeanah & Anders, 1988). In this study sleep was recorded with a video camera and this was compared with parental perception of the child's temperament. Rhythmic, distractible and easy (components of temperament) children, rated by the father, had more mature sleeping patterns (longer sleep duration) and spent less time out of the crib. When the father rated their child as intense (component of temperament) these children also had a longer sleep duration. Arrhythmic children, rated by the mother, had a smaller percentage of active sleep. Overall parental ratings of temperament significantly correlated with sleep variables, but father's perception was correlated with more sleep variables than ratings of the mother. Temperament seems to be interrelated with sleep patterns, but more research is necessary.

Temperament as a moderator

Not every child is influenced the same way while experiencing the same environment (Belsky, Bakermans-Kranenburg & Van IJzendoorn, 2007). Children within the same family can vary in their susceptibility towards certain experiences (Belsky, 1997). This can be the result of differences in temperament, genes or other individual characteristics (Belsky et al., 2007). There is a proposition that some children are more affected by certain rearing experiences than others, in both supportive, positive parenting and unsupportive, negative parenting. The impact of certain types of parenting can have an extra positive effect or an extra negative effect on susceptible children compared to unsusceptible children. Results show that children (M=27.4 months) with a difficult temperament are more susceptible for both negative- and positive discipline than children with an easy temperament (Van Zeijl, Mesman, Stolk, Alink, Van IJzendoorn, Bakermans-Kranenburg, et al., 2007). The same results can be found in childcare (Pluess & Belsky, 2008). Children with a difficult temperament are more affected by both positive - and negative childcare than children with an easy temperament. Temperament can also play an important role in the effectiveness of an intervention (Klein-Velderman, Bakermans-Kranenburg, Juffer & Van IJzendoorn, 2006). An intervention aimed to promote the sensitivity of the mother was most effective only in highly reactive children and their mothers. Temperament is an important factor that can influence a variety of factors in different ways.

The actigraph

The actigraph is an objective measure for recording sleep in a naturalistic setting (Gnidovec, Neubauer & Zidar, 2002). This measure can continually record movements for prolonged periods. There are two widely used types of recorders, the Motionlogger of Ambulatory Monitoring Inc. and the Gaehwiler (Pollak, Stokes & Wagner, 1998). Still, there is not a golden standard for measuring motor activity during sleep.

The validity of the actigraph has been investigated several times (Gnidovec et al., 2002). The validity of the Gaehwiler was investigated with infants of one, three and six months of age. The actigraph results were compared with direct observations of the infant. This type of actigraph is a valid instrument for measuring sleep versus wake states in infants of three and six months. However, the Gaehwiler did not provide valid active versus quiet sleep measurements. Also the validity of the Motionlogger was investigated with one year olds (Sadeh, Acebo, Seifer, Aytur & Carskadon, 1995). The measurements of the actigraph were

compared with direct observations by trained observers. Here the actigraph distinguished between wake and sleep states, but had lower reliability rate for the distinction between active and quiet sleep. When the Gaehwiler and the Motionlogger were compared both were proved valid (Pollak et al., 1998), both types suffered from insensitivity, but the Motionlogger was more sensitive than the Gaehwiler.

Research questions

The influence of smell on the (sleeping) behavior of both babies and adults is apparent (Goubet et al., 2003; MacBurney et al., 2006; Rattaz et al., 2005). Specifically the smell of the own mother influences the behavior of the newborn baby (Marlier & Schaal, 2005; Nishitani et al., 2009). Nevertheless the influence of the smell of the own mother on sleeping- and crying behavior of infants *in daycare* has never been investigated, but similar studies (in the hospital or in the laboratory) have already been done (Russell, 1976; Sullivan & Toubas, 1998). Sullivan and Toubas (1998) investigated if the smell of the own mother has an influence on the crying, sleeping and awake newborn compared to the smell of another mother, a neutral smell or no smell. Results show that newborns increased mouthing when they smelled their own mother, but this was not found when the infants were already asleep. The same result was found in the study of Russell (1976). He investigated whether a newborn can discriminate between the smell of their own mother, the smell of another mother and no smell. Results showed that sleeping newborns cannot discriminate between the three different smells up to six weeks postnatal.

In the current study we will investigate the influence of the smell of the own mother, another mother and a neutral smell on the sleeping behaviour of the infant in daycare, but we will place the object with the different scents in the crib before the baby is put down to sleep. So we can investigate the effect of different smell on *falling* asleep instead of placing an object with different scents in the crib of an already sleeping infant, as was the case in the two previously discussed studies (Russell, 1976; Sullivan & Toubas, 1998). It is important for infants to rest in daycare because they have elevated cortisol levels (Vermeer & Van IJzendoorn, 2006) and sleep regulates cortisol (Larson et al., 1991; Scher et al., 2009). Researchers concluded that there is a significant impact of temperament on different aspects of behavior (Halpern et al., 1998; Keener et al., 1988; Scher et al., 1998). Babies with a difficult temperament had a shorter duration of sleep (Weissbluth & Liu, 1983) and experienced more night wakening (Carey, 1974) than babies with an easy temperament.

In addition to the main effect of temperament on sleep behaviour, temperament may also play a different role. The differential susceptibility proposes that not every child is influenced the same way by the environment (Belsky, 1997; Belsky et al., 2007). Some children are more susceptible to certain experiences than others. This can be the result of differences in temperament: Previous research has shown that children with difficult temperaments are more susceptible to the environment than children with easier temperaments (Klein-Velderman et al., 2006; Van Zeijl et al., 2007).

This led to the following research questions: Do children (3-6 months) sleep better (defined as short time falling asleep, long total duration of sleep, little wake minutes and a good quality of sleep) in daycare if they smell their own mother compared to the smell of another mother or a neutral smell? Previous studies showed that during pain the familiar smell of the own mother has a soothing effect on the child (Goubet et al., 2003; Marlier & Schaal, 2005; Nishitani et al., 2009; Rattaz et al., 2005). Regarding discrimination between different smells during sleeping no differences were found between the smell of the own mother and another mother (Sullivan & Toubas, 1998). In addition, in the study of Russell (1976) sleeping babies could discriminate between the different smells, but only after six weeks of life. In the current study we will test the baby when he/she is falling asleep. It is hypothesized that infants (3-6 months) will sleep better when they smell their own mother compared to the smell of another mother and a neutral smell.

Secondly, do children with a difficult temperament have more trouble sleeping in daycare than children with an easy temperament? Results show that children with a difficult temperament have more trouble sleeping than children with an easy temperament (Carey, 1974; Keener et al., 1988; Weissbluth & Liu, 1983). The hypothesis in this study is that children with a difficult temperament, rated by the mother, have more difficulty sleeping in daycare than children with an easy temperament.

Finally, the following research question will be investigated: Are there differences in the effect of the smell of the own mother, the smell of the other mother and a neutral smell between children with an easy- or difficult temperament? In the study of Pluess and Belsky (2008) children with a difficult temperament were more affected by both positive and negative childcare than children with an easy temperament. That is why it is hypothesized that children with a difficult temperament will benefit the most from the smell of their own mother during sleep, they will sleep better compared to children with an easy temperament.

Methods

Participants

In total 23 babies and their mothers participated in this study. The sample consisted of 15 boys and 8 girls. The average age of the baby during the first scheduled visit was 23.74 weeks (SD=4.71, range=15-33). The average time from first to last visit was 3.36 weeks (SD=3.11, range=0-12). The infants had an average Apgar score of 8.81 (SD=1.69, range=2-10) and 91.3% of the babies had the Dutch nationality. After birth 65.2% of the babies were (mainly) breastfed, 21.7% (mainly) bottle fed and 13.0% were both breastfed and bottle fed. In two weeks prior to the first visit only 34.8% of the babies were breast fed, while 60.9% were bottle fed and 4.3% received both. Most of the babies had spent 1-2 months in daycare (60.9%), 34.8% had spent 3-4 months in daycare and 4.3% only 0-4 weeks. On the question 'Does your child currently have a stable sleeping pattern' 52.2% of the mothers answered 'yes' and 47.8% answered 'no'. Two babies (8.7%) were defined by their mother as having a sleeping problem. The average age of the mother was 33.35 years (SD=2.92, range=28-40) and 91.3% of the participating mothers were Dutch.

Procedure

First contact was made by phone to ask the name of the location manager to whom the brochure with information about the study could be best sent. The brochure with information about the study was sent to several daycare centers in Alphen aan de Rijn, Capelle aan de IJssel, Gouda, Leiden, Leiderdorp, Leidschendam, Oestgeest, Rijnsburg, Voorburg and Zoetermeer (n=47). After one week the location manager was contacted to ask whether they received the brochure and if they wanted to participate in the study. In total 23 daycare centres gave permission to participate in the study. Reasons for not participating were: the video camera in the bedroom, too busy for the group, and too busy because of participation in other studies. A couple of daycare centers that wanted to participate did not have babies in the age range of 3 to 6 months old (n=8), which resulted in a total of 15 participating daycare centers. After consent of the center, brochures with a consent form for the mothers were sent to the daycare center. The location manager gave those to the mothers of babies of 3 to 6 months of age, who were Dutch and who attended daycare at least twice a week. A large number of babies in this age range do not attend daycare twice a week, so babies who went to daycare once a week were also included. Mothers were asked to say on the consent form on which days their baby attended the daycare center. After a week the location manager was called to

ask if they had response from the mothers. If mothers agreed to participate, they were contacted to explain the study in more detail and visits were scheduled. The T-shirts, questionnaires and letter with information about the visits were sent to the mother.

For each baby there are three conditions: (1) *T-shirt of their own mother*, (2) *T-shirt of another mother*, and a (3) *neutral T-shirt*. The order of the T-shirts was assigned randomly, so that the results could not be influenced by habituation to the T-shirts in the bed during sleep in daycare and the amount of time spent in daycare. Mothers were asked to wear the T-shirt for three consecutive nights before the visit and to shower without any soap and shampoo before going to bed. They filled out a questionnaire on the last morning wearing the T-shirt with questions about illness of the mother, illness of the child, if the mother used perfume during the three days of wearing the T-shirt and if she showered without using any soap or shampoo before going to bed. The mothers were asked to bring the T-shirt to the daycare on the morning of the visit. If this was not possible the research assistant went to the mothers' house and picked up the T-shirt. Two babies were paired together, so the mother of baby 1 wore a T-shirt for baby 2, and mother 2 wore a T-shirt for baby 1. If this was not possible another mother with an infant of around the same age wore a T-shirt for three consecutive nights. Several questionnaires were given to the mother about the normal crying and sleeping behavior of the child, demographic characteristics about the family and temperament.

The babies were filmed during the first sleep in daycare with one of the T-shirts put around the mattress of the baby. An actigraph was put around the ankle of the baby for the assessment of sleeping behavior and a decibel meter was put under the tripod of the video camera for monitoring the sound in the environment.

Instruments and materials

T-shirts

The white T-shirts used in this study were 100% cotton. They were all size XL or XXL, so that the T-shirt could be put around the mattress. Before the start of the study all T-shirts were washed with a neutral washing powder (Neutral) and then stored in a zip lock bag. The mothers were asked to put the T-shirt in the zip lock bag every night after wearing the T-shirt, so the smell would not fade or be influenced by other smells.

Actigraph

An actigraph, type MicroMini Motionlogger from Ambulatory Monitoring Inc. (zero crossing mode; this is the most common mode of operation and can be seen as a way of *counting* movements), was put around the ankle of the infant before going to bed. The software Action W was used to score the data (Sadeh algorithm for infants). The following parameters were used in this study: *SLEEP*- total sleep minutes (sleep + light sleep), *AWAKE*- total wake minutes, *WASO*- minutes awake after sleep onset (wake minutes after sleep onset and sleep offset (O-O) interval), *SLAT*- sleep latency (minutes to start of first 20 minute block with more than 19 minutes of sleep) and *SEF*- sleep efficacy (an index of sleep quality), time spent asleep out of the total sleep period ($100 * \text{sleep minutes} / \text{O-O duration}$). A new variable was constructed: *WBS*- wake minutes before falling asleep.

Video's

The babies were filmed during the first sleep of the day in the daycare center. An infrared camera was used for recording the sleeping behavior of the infant. After the data collection all videos were coded, but this data is not used in the current study.

Decibel meter

A decibel meter was used for the recording of sounds in the surrounding of the baby. This meter was placed under the tripod of the camera. Again this data is not used in the current study.

Background questionnaires

Several questionnaires were given to the mothers. Questionnaires A and B were filled out by the mother on the mornings after wearing the first and second T-shirt. The questions were about illness of the child, illness of the mother, perfume use of the mother and shower without any soap and shampoo. Questionnaire 1 assessed background information about the mother and the child. The two remaining questionnaires were about the crying and sleeping behavior of the infant in the past two weeks.

Infant temperament

For the assessment of temperament the Infant Characteristics Questionnaire (ICQ) was used (Bates, 1979). The ICQ consists of 24 items, describing concrete behavior in certain situations. The items were rated on a 5-point scale, ranging from 0 'not true' to 4 'true'. The ICQ consist of four factors (Bates, 1979). A principal component analysis was carried out to derive a general difficultness factor (Bates, 1979; Van Zeijl et al., 2006). The difficultness factor consists of 9 items, as described by Bates (1979). The internal consistency is 0.68 respectively (Van Zeijl, et al., 2006).

Analysis

In the preliminary analysis the distribution of the data is investigated and possible outliers are defined. The variables that are defined as outliers are winsorized so they can be used in further analysis. For the investigation of the stability of sleeping behavior during the three conditions correlations are computed. For the second and third hypothesis the group is divided in two groups using a median split based on the difficultness factor of the ICQ. To investigate if the two groups differ on the background variables several statistical test are done. Independent *t*-test are executed for the variables age, Apgar score and daycare group size to determine whether infants with a difficult temperament differ on these variables from infants with an easy temperament. The same is done for the categorical variables time in daycare (0-4 weeks, 1-2 months and 3-4 months), gender, type of feeding from birth, type of feeding past 2 weeks, pacifier, sleeping pattern, sleeping problems, and crying during the day past week with a chi-square test.

Effect of smell on the sleeping behavior of the infant

The first hypothesis, infants (3-6 months) will sleep better when they smell their own mother compared to the smell of another mother and a neutral smell, is tested using six repeated measures ANOVAs with the variables *SLEEP*, *AWAKE*, *WASO*, *SLAT*, *SEF* and *WES* per type of T-shirt (own mother, other mother, neutral) as within-subjects factors.

Temperament and sleep

For the second hypothesis the group is divided in two groups using a median split based on difficultness score on the ICQ. Principal component analysis was done to compute this difficultness score (De Schipper, Tavecchio, Van IJzendoorn & Linting, 2003; Stams,

Juffer & Van IJzendoorn. 2002). One group is defined as ‘easy’ and the other group is defined as ‘difficult’. Six repeated measures ANOVAs are computed to test the main effect of temperament with the following variables: total sleep minutes (*SLEEP*), total wake minutes (*AWAKE*), minutes awake after sleep onset (*WASO*), sleep latency (*SLAT*) sleep efficacy (*SEF*) and total time before falling asleep (*WBS*).

The moderating effect of temperament

An interaction effect between temperament and type of smell is carried out using six repeated measures ANOVAs with the same variables as in the former analysis. These analyses will indicate whether children with a difficult temperament will benefit more from the T-shirt with the smell of their own mother than children with an easy temperament.

Results

Preliminary analysis

One subject was excluded from the analyses because of errors in the procedure: the baby fell asleep in the arms of the caregiver and was then put to bed. Z-scores were calculated to define possible outliers. A score was defined as an outlier if it was higher than 3.29 and lower than -3.29. There were three outliers on the following variables: wake minutes in the neutral condition, wake after sleep onset in the own mother condition and in the neutral condition. The outliers were winsorized using the 25th percentile for the lowest score and the 75th percentile for the highest score (Grace & Sawilsky, 2009). The normality of the data was checked for all variables (Table 1). A variable is defined as not normal if the skewness is smaller than -3 or larger than 3. All sleep variables are normally distributed.

Table 1: Mean, standard deviation, skewness and kurtosis sleep variables.

	M	SD	Skewness		Kurtosis	
			<i>Sk</i>	<i>SE</i>	<i>Ku</i>	<i>SE</i>
MT <i>SLEEP</i>	53.50	31.47	1.07	0.49	0.48	0.95
MT <i>SEF</i>	89.41	19.26	-2.13	0.49	4.18	0.95
MT <i>SLAT</i>	20.95	16.66	1.26	0.49	0.76	0.95
MT <i>WAKE</i>	47.09	28.64	1.06	0.49	1.40	0.95
MT <i>WASO</i>	12.27	26.87	2.39	0.49	5.53	0.95
MT <i>WBS</i>	19.41	15.23	1.28	0.49	0.79	0.95
OT <i>SLEEP</i>	49.82	25.27	0.12	0.49	-1.37	0.95
OT <i>SEF</i>	95.84	7.24	-1.68	0.50	1.61	0.97
OT <i>SLAT</i>	15.05	10.45	1.42	0.50	1.78	0.97
OT <i>WAKE</i>	35.27	19.59	0.94	0.49	0.33	0.95
OT <i>WASO</i>	3.57	6.79	2.14	0.50	3.97	0.97
OT <i>WBS</i>	14.77	10.73	1.20	0.49	0.92	0.95
NT <i>SLEEP</i>	53.73	34.28	1.16	0.49	0.38	0.95
NT <i>SEF</i>	93.31	13.18	-2.30	0.49	4.50	0.95
NT <i>SLAT</i>	19.23	15.03	0.87	0.49	-0.60	0.95
NT <i>WAKE</i>	38.77	26.26	0.15	0.49	-1.48	0.95
NT <i>WASO</i>	7.68	18.36	2.93	0.49	8.62	0.95
NT <i>WBS</i>	14.77	12.15	1.10	0.49	-0.21	0.95

* *MT=own mother T-shirt, OT= other mother T-shirt and NT= neutral T-shirt.*

Stability of sleeping behaviour

To test the stability of sleeping behaviour correlations were computed for the three conditions. Several significant correlations were found (Table 2). There was a significant association between time before falling asleep across the three conditions ($r=0.44$, $p<0.05$; $r=0.54$, $p<0.05$; $r=0.50$, $p<0.05$). Time before falling asleep was stable for the three conditions. For the variables *WAKE* (total wake minutes) none of the correlations were significant and for the variable *SLEEP* (total sleep minutes), *SEF* (sleep efficacy) and *WASO* (wake after sleep onset) the correlation was only significant between the other mother T-shirt and the neutral T-shirt. Finally the variable *SLAT* (sleep latency) was only significant between the mother T-shirt and the other mother T-shirt and the mother T-shirt and the neutral T-shirt.

Table 2: Correlation between sleep variables during the different conditions

	<i>Mother – Other mother</i>	<i>Mother - Neutral</i>	<i>Other mother - Neutral</i>
<i>Time before falling asleep (WBS)</i>	.44*	.54*	.50*
<i>Total wake time (WAKE)</i>	.34	.22	.27
<i>Total sleep time (SLEEP)</i>	-.38	-.37	.67*
<i>Sleep efficacy (SEF)</i>	-.03	-.25	.80*
<i>Sleep latency (SLAT)</i>	.45*	.57*	.17
<i>Wake after sleep onset (WASO)</i>	.06	-.24	.51*

* $p<0.05$

Covariates

For the analysis with temperament the group was divided into two groups using a median split based on the difficultness factor from the ICQ (median=1.39). One group was defined as ‘easy temperament’ (n=11) and the other group was defined as ‘difficult temperament’ (n=11). The two groups did not differ in age ($t[20]= 0.84, p=0.41$) and Apgar score ($t[19]= 1.34, p=0.20$). There was a significant difference between the two groups on the variable group size in daycare ($t[17]= 3.56, p<0.01$). Infants with an easy temperament were staying in bigger groups in daycare than infants with a difficult temperament. This variable was used as covariate in further analyses with temperament. For the categorical variables, chi-square tests were calculated to test the difference between the two temperament groups on the following variables: time in daycare ($p=0.09$), gender ($p=0.56$), type of feeding from birth ($p=0.32$), type of feeding past two weeks ($p=0.09$), pacifier ($p=0.72$), sleeping pattern ($p=0.09$), sleeping problems ($p=0.31$) and crying during past two weeks ($p=0.35$). None of these categorical variables differed significantly between the two groups.

In addition, correlations between the sleep variables and all the background variables were tested. Significant correlations were found between number of sleep minutes in the own mother T-shirt condition and Apgar score ($r=-0.50, p<0.05$) and wake before sleep onset in the other mother T-shirt condition and if the mother wore perfume during this condition ($r=-0.51, p<0.05$). Illness of the child is significantly with sleep latency ($t[20]= -2.40, p<0.05$) and wake before sleep onset ($t[20]= -2.64, p<0.05$) in the own mother T-shirt condition. So illness of the child will be used as a covariate in the further analyses.

Effect of smell on sleeping behaviour

To test the main effect of type of smell on sleeping behaviour of the infant six repeated measures ANOVAs were done with the variables *SLEEP* (total sleep minutes), *AWAKE* (total wake minutes), *WASO* (wake after sleep onset), *SLAT* (sleep latency), *SEF* (sleep efficacy) and *WBS* (wake before sleep onset). For the variable total sleep minutes (*SLEEP*) a marginally significant effect was found (Figure 1) ($F[1.30,20.86]=3.12, p=0.08$). Infants slept longer in the neutral T-shirt condition (M=2.24, SD=0.22) compared to the own mother T-shirt condition (M=2.23, SD=0.22) and the other mother T-shirt condition (M=2.23, SD=0.22). No significant effects were found for the number of wake minutes ($F[2,32]=0.06, p=0.94$), wake minutes after sleep onset ($F[2,32]=0.49, p=0.62$), sleep latency ($F[2,32]=0.28,$

$p=0.73$), sleep efficacy ($F[1.12,17.86]=0.65$, $p=0.48$) and wake minutes before falling asleep ($F[2,32]=0.38$, $p=0.69$).

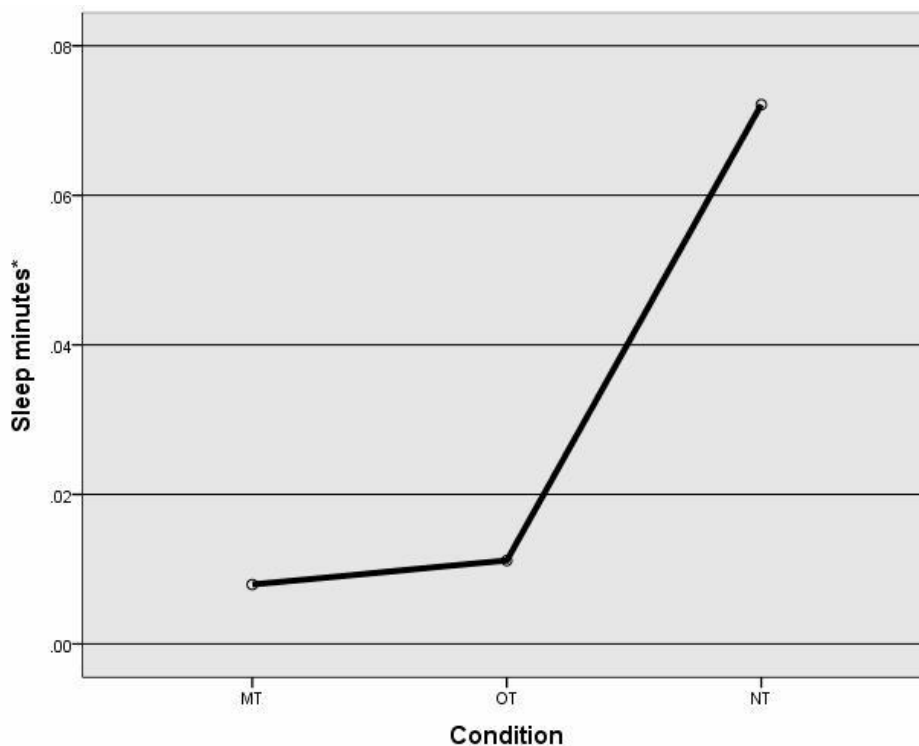


Figure 1: Repeated measures ANOVA total sleep minutes (SLEEP)

*Z-scores

Temperament and sleep

Six repeated measures ANOVAs with temperament as factor were done to test the main effect of temperament on infant sleep. No significant effects were found: *AWAKE* ($F[2,22]=0.44$, $p=0.65$), *WASO* ($F[2,22]=1.52$, $p=0.24$), *SLAT* ($F[2,22]=0.06$, $p=0.95$), *SEF* ($F[1.16,12.77]=1.55$, $p=0.24$) and *WBS* ($F[2,22]=0.30$, $p=0.73$). A marginally significant main effect was found for the variable *SLEEP* (Figure 2) ($F[1.17,12.88]=3.72$, $p=0.07$). Infants with a difficult temperament ($M=0.20$, $SD=0.28$) slept longer than infants with an easy temperament ($M=-0.04$, $SD=0.25$).

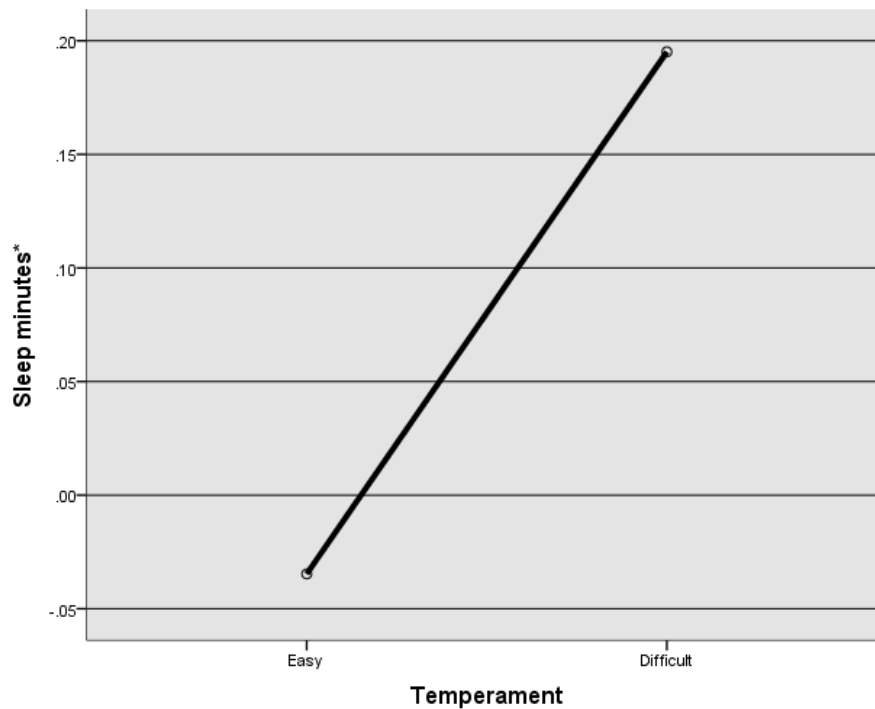


Figure 2: Repeated measures ANOVA sleep minutes and temperament.

*Z-scores

Moderating effect of temperament

To investigate whether babies with a difficult temperament would benefit more from the T-shirt with the smell of their own mother than babies with an easy temperament six repeated measures ANOVAs were performed with the same variables as in the former analysis. No significant interaction effects were found for all variables: *SLEEP* ($F[1.17,12.88]=0.24$, $p=0.67$), *AWAKE* ($F[2,22]=0.29$, $p=0.75$), *WASO* ($F[2,22]=1.23$, $p=0.31$), *SLAT* ($F[2,22]=0.22$, $p=0.80$), *SEF* ($F[1.16,12.77]=0.06$, $p=0.84$) and *WBS* ($F[2,22]=0.93$, $p=0.41$).

Discussion

In this study three research questions were investigated: (1) do children sleep better (defined as short time falling asleep, long total duration of sleep, little wake minutes and a good quality of sleep) in daycare if they smell their own mother compared to the smell of another mother or a neutral smell? (2) do children with a difficult temperament have more trouble sleeping in daycare than children with an easy temperament? and (3) are there differences in the effect of the smell of the own mother, the smell of the other mother and a neutral smell between children with an easy- or difficult temperament? In this study Dutch infants who attend daycare at least one day a week were filmed three times during their first sleep in daycare: once with a T-shirt worn by their own mother (MT), once with a T-shirt worn by another mother (OT) and once with a neutral T-shirt (NT). The infants wore an Actigraph around their ankle for the assessment of their sleeping behaviour.

In daycare a lot of infants have elevated cortisol levels, indicating high levels of stress (Vermeer & Van IJzendoorn, 2006). It is important for the regulation of stress levels to have sufficient sleep, especially for children younger than 36 months. The familiar smell of the own mother can have a soothing effect on the child during pain (Goubet et al., 2003; Rattaz et al., 2005), but can it also have a soothing effect during sleep? If the smell of their own mother can help the infants sleep better in daycare, they will have lower stress levels which is better for their development later in life (Vermeer & Van IJzendoorn, 2006).

Results showed no significant effect of type of smell on sleeping behaviour. However, a *marginal* effect was found for the variable total sleep minutes (*SLEEP*), infants slept longer in the neutral T-shirt condition compared to the own mother T-shirt condition and the other mother T-shirt condition. Beforehand, it was hypothesized that infants will sleep better when they smelled their own mother compared to the other two conditions. In the study of Sullivan and Toubas (1998) results showed that when infants were already asleep, there was no significant effect between the smell of the own mother, the smell of another mother, a neutral smell and no smell in mouthing of the child. The study that *did* find an effect with sleeping infants used head orientation as a construct to examine the preference for a certain smell (Russell, 1976). So in these two studies different constructs were used to determine the preference for a certain smell compared to the present study where quality of sleep by counting sleep- and awake minutes using an actigraph was assessed. Maybe this unexpected result, that infants sleep longer when they smell a neutral smell, is true when the index for

sleep is counting sleep- and awake minutes (quality of sleep), but when mouthing and/or head orientation (preference for a certain smell) is assessed a different result will appear.

Perhaps the smell of the own mother only has a soothing effect on the infants' crying behavior. However, when crying is taken into the analysis maybe a different result will appear. Most of the previous studies investigating the effects of certain smells on infants were done during a pain response (Nishitani et al., 2009; Rattaz et al., 2005). In these studies the results showed that infants stopped crying sooner when they smelled their own mother compared to other smells. Especially the familiarity with a certain smell can have a soothing effect during pain (Goubet et al., 2003; Rattaz et al., 2005). However, when babies were sleeping no differences were found between the smell of their own mother and the smell of another mother (Sullivan & Toubas, 1998), as in this study. When babies are sleeping they are not distressed, so they do not need soothing. It is a different story when the babies are crying, because then they are distressed and in need of soothing. This can be an explanation for the fact that results are often found when babies are crying (Nishitani et al., 2009), but not when they are asleep (Sullivan & Toubas, 1998). So maybe the familiar smell of their own mother can help them terminated crying sooner, but this is beyond the scope of this research.

In this study no significant effects were found of type of smell (own mother, another mother, and a neutral smell) on the sleeping behavior of infants. However, a lot of studies showed that children do respond differently to the smell of their own mother compared to other smells, especially during pain (Cernoch & Porter, 1985; Porter et al., 1991; Russell, 1976). Young infants (even newborns) can discriminate between the smell of their own mother compared to the smell of another mother. But inconsistent results have been found when babies are formula-fed (Cernoch & Porter, 1985). Especially the familiarity with a certain smell can have a soothing effect on the infant (Goubet et al., 2003; Rattaz et al., 2005). The sample in this study consists of a large number of babies that were breastfed after birth (n=15), but they were not matched with other breastfed babies for the exchange of the T-shirts. A baby who was breastfed could have been given a T-shirt from a mother who bottle-fed her baby. Infants who were breastfed are not familiar with the smell of formula-milk and the same is true the other way around; infants who are formula-fed are not familiarized with the smell of the mother milk of another mother. Previous research showed that the familiarity with a certain smell has an influence on the infants (Goubet et al., 2003; Rattaz et al., 2005). This can be an explanation why no significant differences are found between the three conditions.

Secondly, all infants participating in this study went to daycare for a minimum of one day a week. When babies are more familiarized with sleeping in daycare, it can be expected that they will benefit more from the smell of their own mother than the infants in this study. All infants in this study had only spent one to two months in daycare. If they attend daycare only once a week, as most infants in this study, it can be said that they are not used to the circumstances in daycare and their caregivers. Going to daycare is new to them, so they will not benefit from the smell of their own mother. Many other factors influence sleeping behavior of infants who are not yet familiarized with the setting, so it is difficult to find an effect. Once they are familiarized with the daycare and their caregivers, an effect can be expected. So it is important to further investigate the effect of the smell of the own mother in older children, who have attended daycare for a longer period of time. Finally, the lack of significant effects in this study can be explained by the relatively small sample size as compared to other studies (Nishitani et al., 2009; Rattaz et al., 2005). Studies that did find a result had larger samples, namely 48 (Nishitani et al., 2009), 51 (Goubet et al., 2003), 59 (Rattaz et al., 2005), 81 (Sullivan & Toubas, 1998). Small samples have a low power, so effects are more difficult to detect (Moore & McCabe, 2006).

No main effect of temperament was found in this study. This is in line with part of the literature where no effects of temperament on sleep were found (Ottoni et al., 2010; Scher et al., 1998), but contradictory to the other part of the literature where significant associations between sleep and temperament were found (Carey, 1974; Keener et al., 1988; Weissbluth & Liu, 1983). In this study temperament was assessed using a questionnaire filled out by the mother. A difficultness score was calculated to define temperament, with a highest score of four. In this sample the difficultness score had a small range (0.67-2.44). Infants with a difficultness factor higher than 1.39 (median) were defined as difficult, but in fact these infants still scored relatively low on difficultness. So the infants who were defined as having a difficult temperament were the most difficult children of the *sample* in this study and were in terms of the population not really difficult. So the fact that no significant results were found, can be caused by the small range in temperament. It is not surprising that no significant results were found, because all infants in this sample were 'easy'. When a sample with infants with a *true* difficult temperament are included (median higher than 3) results may be different. However a *marginally* significant effect was found on the variable *SLEEP*, where infants with a difficult temperament slept longer than infants with an easy temperament. This result is unexpected, because it was hypothesized that children with a difficult temperament will have

more difficulty sleeping in daycare than children with an easy temperament. Sleep and awake states are influenced by the behavior of the infant itself (Halpern et al., 1994), and also by external factors. So this marginal effect can be caused by *confounding* factors, both internally and externally, that influences the sleeping behavior of the infant, namely overall sleeping pattern, quality of sleep on the previous day, biological maturity and the relationship between the infant and the caregiver (Keener et al., 1988).

No moderating effect was found of temperament in the relation between type of smell and the sleeping behaviour of the child. It is often found that children with a difficult temperament are more susceptible to their environment than children with an easy temperament (Klein-Velderman et al., 2006; Pluess & Belsky, 2008; Van Zeijl et al., 2007). In this study it was hypothesized that children with a difficult temperament will benefit the most from the smell of their own mother during sleep. They will sleep better with their mother's smell compared to children with an easy temperament. Again this hypothesis was rejected. Temperament is based on several dimensions, namely activity, rhythmicity, arousal, irritability, state regulation and soothability (Keener et al., 1988). Some dimensions of temperament will have more of an influence on sleeping behavior than others, like activity, rhythmicity, arousal, and soothability. It may be the case that no moderating effect of temperament (as a whole) was found, because only some dimensions of temperament have an influence on sleeping behavior of infants. When only these dimensions will be taken into account, a moderating effect may be found.

The results of this study show that infants did not sleep better when they smelled their own mother compared to the smell of another mother or a neutral smell. No differences were found between infants with a difficult and an easy temperament. It is important to further investigate the effect of smell the own mother on the sleeping behavior of the child in daycare, because sleep is important for the regulation of cortisol and indirectly for their later development (Vermeer & Van IJzendoorn, 2006). It is important that this will be done with a larger sample and with older children, who are attending daycare at least two days a week or more and for a longer period of time. Because an object with the smell of the mother can work as an intervention in daycare, infants may sleep better and their stress may be regulated.

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