Attachment Behavior in Rats

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Cover: Two way choice cages as used in experiments described in Chapters 3 and 4.

Design & Layout: Hein Sigling.

Print: Uitgeverij Box Press, Oisterwijk

ISBN: 978-90-393-5007-2

Attachment behavior in rats

Hechtingsgedrag bij de rat

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van Doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 12 februari 2009 des middags te 16.15 uur

door

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geboren op 20 juni 1956
te Amstelveen.

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CHAPTER 1

Introduction to attachment theory and its relevance for psychopathology

In the first part (I.1) of this chapter, a short history of attachment theory is presented through the work of three of the most important contributors. This is followed by an overview of the empirical evidence for attachment theory through a discussion of 5 important hypotheses. In the second part (I.2), the clinical relevance of attachment theory is investigated in a review of the association between attachment variables and psychiatric disorders.

I.1 History of attachment theory

I.1.A John Bowlby

In 1948 the Social Commission of the United Nations decided to study the needs of homeless children. The World Health Organization wanted to contribute with a study of their mental health and British child psychiatrist and psychoanalyst John Bowlby was appointed to undertake it. Bowlby was then head of the Children and Family department of the Tavistock Clinic in London, one of the world's most renowned psycho-analytically oriented mental health institutes. In 1944 he had published the monograph 'Forty-four juvenile thieves: their characteristics and home life', in which he described in what was for those days a very methodically way of observing the lack of care and the early separations those boys had encountered. For the WHO, Bowlby studied the literature on maternal deprivation and visited institutions and experts in Europe and the United States. This resulted in the 1951 WHO monograph 'Maternal care and mental health'. In chapter 1 Bowlby states that: '*what is believed to be essential for mental health is that the infant and young child should experience a warm, intimate, and continuous relationship with his mother (or permanent mother-substitute) in which both find satisfaction and enjoyment.'....' It is this complex, rich, and rewarding relationship with the mother in the early years, varied in countless ways by relations with the father and the siblings, that child psychiatrists and many others now believe to underlie the development of character and of mental health.'* This statement reflects the radical break between the intrapsychic orientation of classical psychoanalysis and the dyadic orientation of attachment theory, then in the making. The reason for this break becomes clear when reading the rest of the monograph. The

positivistic focus on measurable behavior in children and the correlation with factual circumstances like presence or absence of a mother-figure had forced Bowlby away from classical psychoanalytic theory and had compelled him to develop a theory that is dyadic in nature.

With regard to the term attachment: on page 31 of the monograph, Bowlby cites an article by Powdermaker et al., published in 1937: *'Later it became clear that the feature common to them was an inability to make a real transference to any member of the staff. There might seem to be a good contact but it invariably proved to be superficial...There might be protestations of interest and a boisterous show of affection, but there was little or no evidence of any real attachment having been made. In going over their previous history, this same feature was outstanding... [These girls] have apparently had no opportunity to have a libidinal relationship in early childhood [and] seem to have little or no capacity to enter into an emotional relation with another person or with a group'*. The article was, together with an article by Levy (cited by Bowlby) from the same year, one of the first of a series during the (late) 1930s and 1940s, in which the effect of maternal deprivation upon socio-emotional development was noted and investigated. It was probably the first article in which the term 'attachment' was used in its contemporary psychosocial meaning, albeit in a context of many other terms describing more or less the same phenomenon: 'real transference', 'good contact', 'libidinal relationship' and 'emotional relation'.

In the quotation from Powdermaker et al., the term 'attachment' is used to describe relationships with the staff of an institution after maternal deprivation had taken place. Bowlby himself uses the term to describe primary infant-caregiver relations on page 47 of the WHO monograph when he mentions three different experiences that can 'produce the affectionless and psychopathic character'. One of these is: *'lack of any opportunity for forming an attachment to a mother figure during the first three years'* suggesting that if the opportunity is given, the child will form an attachment. This is a prelude to his later work, in which the inclination to form attachments is seen as an innate quality that has been, through its adaptive advantages, strengthened during the course of evolution. Another important aspect of attachment theory, the inter-generational transfer of pathological attachments, is introduced on page 69 as follows: '*This vicious circle is the most serious aspect of the problem and one to which this report will constantly revert*'.

Bowlby belonged to an avant-garde group of psychoanalysts that showed growing discontent from the 1930s with the orientation on fantasy and the individual child that then prevailed. This discontent was recorded in writing, especially in the 1950s. Central figures in this development were Fairbairn, Balint, Winnicott and Kernberg. They emphasized the importance of the relationship with the mother, but continued theorizing along Freudian lines of secondary drive-theory and economic and structural perspectives. The emphasis is on real-life experience with a comparatively moderate influence of intrapsychic mechanisms which is so central to attachment theory is lacking in these works.

In 1969 Bowlby published the first part of his trilogy Attachment and Loss: Attachment. Here, in which he developed a theory of interactional motivation and behavior control based on the principles of evolution theory, cybernetics and cognitive psychology. The Attachment and Loss trilogy is a work with a very strong emphasis on scientific evidence, stemming from observational studies of infant-maternal interactions as well as from comparative physiology. Bowlby first stressed the development of behavioral and motivational systems in a specific "environment of evolutionary adaptedness", when humans were hunter-gatherers. Bowlby was a great admirer of Charles Darwin and even published a biography about him (Bowlby 1991). In the environment of evolutionary adaptedness, attachment behavior was believed to have selective advantages in promoting proximity to the mother, and thus promoting safety. In the second edition of Attachment in 1983, Bowlby incorporated newer theories about the gene as the principal object of natural selection, emphasizing that promoting safety can only have selective advantages if followed by greater reproductive success.
The attachment behavioral system is clearly distinct from other motivational systems that are important for survival, like the feeding system, and takes priority over those others when needed. Bowlby assumed that Freudian secondary drive theory (according to which the libidinal investment in the mother is secondary to the contact with her in the context of feeding) is incorrect. The attachment relationship between the child and the primary care-giver is the result of a period of interaction between the attachment behavior of the infant and the care-giver's response to it, which only after six months results in a specific bond. The first year is so important in developing these specific attachments that it can be called a sensitive period, after

which the establishment of attachments is much more problematic. Bowlby emphasizes, however, that the sensitive period and the rigidity afterwards are considerably less than in imprinting in birds, from which the idea of the sensitive period originates. Based on observational studies, Bowlby describes the three phases in the child's behavior when confronted with the absence of the primary care-giver: protest, despair and detachment. Initially the child will cry and be angry, restless, hyperactive and easy irritable. After a few hours to a week, the second phase starts: the child will turn inwards, lose interest in the surroundings and in play and will behave in a depressed way, with moaning and sobbing. In the third phase, after a few weeks to a few months, normal behavior and mood seem to return, but the child maintains a detached attitude towards care-givers and may not react at all if the primary care-giver returns.
A crucial aspect of attachment theory is formed by the working models. These are structured and relatively robust cognitions in which experiences with attachment figures are condensed. A child constructs a working model of the self, and its own ability in promoting proximity to the attachment figure, and a working model of the other, in which expectancies of the response of the significant other to the child's attachment behavior are stored. These working models influence the perception of intimate relations and the behavior in them throughout life.

In the second part of the trilogy, 'Separation' (Bowlby 1973), a theory of anxiety is developed in which there is not only place for the more profane fear-inducing signals like loud noises, animals, darkness etc, but also for signals that indicate (the threat of) absence of the attachment figure. This fear of separation is counterbalanced by an innate tendency to explore. The organism strives towards homeostasis by avoiding excessive domination of either of the two tendencies. With the attachment and the explorational system, Bowlby distinguishes the care-giving and the sexual mating system as the four behavioral systems that promote survival and procreation. These systems are goal-directed. For the attachment system, the goal is 'felt security'. As long as this goal is not reached, the attachment system stays active. In 'Separation' Bowlby also expands on the nature of the working models and accounts for inter-generational transmission of attachment behavior.

The third part of the trilogy, ‘Loss’ (Bowlby 1980), deals with the effect of the loss of an attachment figure, especially in children. He describes in further detail the phases of protest, despair and detachment that occur in reaction to loss. Bowlby proposes that depression in adults may be a variant of the energy-saving despair phase that promoted survival in children separated from their attachment figure in the environment of evolutionary adaptedness. In ‘Loss’, Bowlby also describes how the individual deals with conflicting working models or with conflicts between the working model and other types of information. The processes of defensive exclusion of information leading to dissociation and denial that occur, for instance, in pathological mourning are elucidated.

In 1987 Bowlby published 'A Secure Base: Clinical Applications of Attachment Theory'. This is a very practical and readable summary and an update of his earlier work, together with clinical examples and suggestions. In ‘A Secure Base’, Bowlby reduces the emphasis on the mother as the primary attachment figure and stresses that other primary care-givers can have the same role and that multiple attachments are formed during development. He also adjusts his views on the degree of permanence of the experiences in the first year and allows for a more developmental perspective of internal working models and their influence on personality. The more dysfunctional the early attachment experiences were, the more rigid the working models will be throughout life.
With regard to clinical phenomena, he argues that violence in families stems from misdirected and ill-expressed anger that has a functional origin within the attachment system. With amnesia and multiple personality disorder as examples, he describes the role of defensive information-handling strategies in psychopathology. Finally, he describes the influence of attachment experiences on the process of psychotherapy, the clues that behavior in therapy can give about these experiences and the importance of altering working models for effective therapy.

I.1.B Mary Ainsworth

Mary Ainsworth was an American psychologist who was at university in Toronto. In 1940 she wrote her doctoral dissertation on the subject of security within the family and coined the term ‘a secure base’

which 47 years later became the title of one of John Bowlby's books. In the early 1950s she worked as a researcher in Bowlby's department at the Tavistock Clinic in London and acquainted herself with the observational rigor there. When her husband's work took them to Uganda in 1955 and 1956, she started to observe Ugandan mother-child dyads in their homes (Ainsworth, 1967). In this study, 26 mothers and their children were followed for 12 months and their interaction was described meticulously, resulting in classification of the children as securely or insecurely attached. After returning to the US, she started to work at Johns Hopkins University in Baltimore. There, she performed a replication study of her Ugandan work by again observing 26 mothers with their children in their homes. Each dyad was observed for at least 72 hours. In order to standardize the context and organize the occurrence of stressors that should activate the attachment system, she also developed a laboratory procedure called 'The Strange Situation' (Ainsworth 1978). In 'The Strange Situation', children between 12 and 24 months are subjected to a sequence of interactions with, and separations from, their mother and a stranger (see paragraph 1.2.A.). The behavior of the child in this Strange Situation is then classified as secure, insecure-avoidant or insecure-ambivalent. Both the attachment classification system and the Strange Situation have been invaluable in standardizing attachment research and form the basis of thousands of publications in developmental psychology and psychopathology.

I.1.C Mary Main

Mary Main was a student of Mary Ainsworth in Baltimore in the early seventies. She later moved to the University of California at Berkeley and, with her co-workers, developed the 'Adult Attachment Interview' (George, Kaplan & Main 1985), see paragraph I.2.A. The 'Adult Attachment Interview' was developed to measure attachment typology on the basis of aspects of structure and content of a person's narrative about his or her parents. The AAI is a time-consuming instrument. A minimum of one hour of interviewing, approximately 4 hours of verbatim transcription and 3 hours of coding are required. Numerous attempts have been made to develop instruments that were less time-consuming, but the AAI remains the most thorough instrument for measurement of attachment in adults and has a status that is comparable to the Strange Situation in infants. The AAI has been used

in hundreds of studies and has been enormously important for the scientific progress of attachment theory.
Mary Main was also responsible for redefining a residual category in the attachment classification of 'The Strange Situation' (Main & Solomon 1986). This category was previously seen as unclassifiable, because the specific secure, ambivalent or dismissing (see 1.2.A) patterns were not present. These children showed different kinds of contradictory or unproductive attachment behavior and were labeled disorganized or disoriented. Main and co-workers discovered that the disorganized category was over-represented in a group of children that had experienced frightening behavior on the part of the parent. The frightening behavior was often the result of unresolved traumatic experiences in the parent (Main & Hesse 1990; Schuengel, Bakermans-Kranenburg & van IJzendoorn 1999). The disorganized/disoriented category is now generally accepted and is the category that is most clearly linked with psychopathology (van IJzendoorn, Schuengel & Bakermans-Kranenburg 1999; Zeanah, Keyes & Settles 2003), although predominantly in children.

1.2 A Empirical evidence for attachment theory

A number of central expectations of attachment theory are discussed in this paragraph.

1) Because of its evolutionary history, human attachment behavior should have a specific neurobiological substrate, preferably comparable to that in other mammals.

Polan & Hofer (1999) review the parallels between humans and lower mammals in the determinants of development of preference for the mother. They come to the conclusion that there is a striking similarity with respect to the development of olfactorial preferences, the role of suckling, prenatal learning of maternal stimuli and separation responses.
With regard to neuroanatomy, Schore has devoted numerous publications (e.g. 2002; 2005) to his theory that the attachment control system is situated in the right orbitofrontal cortex. The theory is based on a wealth of data regarding brain development during the first few years of life, development of emotional and motivational control, connections with the limbic system, the autonomic nerve system and

the reticular formation etc. In recent years, research has become available that correlates parts of the human brain to attachment, for instance through the use of imaging procedures while performing attachment-related tasks. Bartels & Zeki (2000; 2004) have used fMRI scans to measure the brain activity of people who were deeply in love while viewing a photograph of their loved one, and of mothers viewing a photograph of their child. Romantic love bilaterally highlighted foci in the medial insula and the anterior cingulate cortex and, subcortically, in the caudate nucleus and the putamen. Maternal love also activated the insula and the anterior cingulate, with subcortical activation of the dorsal head of the caudate nucleus, globus pallidus, peri-aquaductal grey and right substantia nigra. In both romantic and maternal love there was de-activation of regions associated with criticism, social evaluation and negative affect. Nitschke et al. (2004) found bilateral activation of the orbitofrontal cortex in mothers viewing photographs of their own children. Ramasubbu et al. (2007) compared fMRI reactions in volunteer women with adequate early life experiences with photographs of their mothers, close female friends and female strangers. Activation of the ventromedial prefrontal cortex/anterior cingulate complex distinguished the reaction to the face of the mother from the reaction to the face of the close friend. Fisher et al. (2006) found activation of the right ventral tegmental area and right postero-dorsal body of the caudate nucleus in romantic love. Gillath et al. (2005) asked 20 women to think about positive and negative relationship scenarios during fMRI. When thinking about negative scenarios there was a positive correlation between attachment anxiety and activity of emotion-related brain areas and a negative relationship with emotion-regulating brain areas like the orbitofrontal cortex. Participants high on avoidance showed less de-activation than less avoidant participants in two brain regions (subcallosal cingulate cortex and lateral prefrontal cortex). Buchheim et al. (2006) found that there was an increase in activation of medial temporal structures when showing pictures related to attachment trauma, but only in unresolved subjects and not in securely attached subjects.

With regard to neuro-endocrinological research, Diamond & Hicks (2004) review the relationship between early attachment experiences and the development of stress regulatory systems. They conclude that there is evidence of a negative influence of insecure attachment on the corticotropin-releasing factor (CRF), hypothalamic pituitary adrenocortical (HPA) axis and vagal tone leading to reduced ability to

cope with stress later in life. Promising research in animals (see Chapter 2) with regard to the involvement of neuropeptides in attachment-like behavior has recently been followed up in humans. There are reports that plasma oxytocin levels are positively correlated with trust (Zak et al. 2005), frequency of hugging with the partner (Light et al. 2005) and social dependency (Uvnas-Moberg et al. 1991). Fries et al. (2005) studied a group of adopted children that spent the first years of their lives in institutions under circumstances of severe neglect and compared them with biological children of households that were similar to the adoptive families. They report that a difference in urinary oxytocin level between these two groups became almost significant 15 minutes after an interaction session with their (adoptive) mother. There was a basal difference in urinary vasopressin that disappeared after the interaction. Intranasal administration of oxytocin can increase the willingness to take social risks in interpersonal interactions (Kosfeld et al. 2005), increases generosity in monetary transactions (Zak et al. 2007) and it improves the recognition of positive facial expressions, while slowing down the recognition of negative facial expressions (Di Simplicio et al. 2008). On the other hand, Turner et al. (2002) did not find reliable oxytocin level alteration as a result of induction of positive or negative emotions related to close personal relationships. In a more specific attachment-related context, recent studies have shown interesting correlations between oxytocin and attachment measures. In romantically unconnected adults, a positive representation of the bond with the parents is positively correlated with oxytocin levels (Gordon et al. 2008). Oxytocin levels during pregnancy are positively correlated with post-natal indices of maternal-infant attachment (Feldman et al. 2007).

In conclusion, there is insufficient evidence to link specific parts of the brain to attachment. Especially, there is a lack of research differentiating activation of brain regions in an attachment context from activation as a result of other positive or negative emotions. Neuroendocrinological studies are growing in number and are gradually becoming sufficiently rigorous. Oxytocin, although careful interpretation is needed because of its role in stress, is emerging as an important factor in attachment.

2) As the attachment (in)security is the consequence of dyadic processes, the relative influence of genes should be limited. Also, different attachment relations should be possible with different people, e.g. attachment classification with the mother can differ from the attachment classification with the father.

There are a few studies that link certain genes to attachment behavior. Lakatos et al. (2000) first reported an association between the DRD4 exon III 7-repeat polymorphism and disorganized attachment. They subsequently reported (Lakatos et al. 2002) that in the same group of children, the -521 C/T single nucleotide polymorphism (SNP) on the same dopamine 4 receptor gene increased the risk of disorganized attachment in the presence of the DRD4 exon III 7-repeat polymorphism. The combination of both would lead to a 10-fold increase in the odds ratio for disorganized attachment. Bakermans-Kranenburg & van IJzendoorn (2004a) did not find this association in a larger group, even when including the DNA of the group of children on whom Lakatos et al., reported. Lakatos and co-workers (Gervai et al. 2005) consequently published a study that showed transmission disequilibrium of the T.7 haplotype of the DRD4 receptor. The T.7 haplotype contains both the 7-repeat polymorphism and the –521 SNP and non-transmission had a protective effect against disorganized attachment. Van IJzendoorn & Bakermans-Kranenburg (2006) recently reported that there was a moderating effect of the 7- repeat polymorphism on the influence of maternal unresolved loss or trauma on the development of disorganized attachment. Maternal unresolved loss or trauma only caused disorganized attachment in the presence of the 7-repeat polymorphism, leading to an 18.8-fold increase in the chance of disorganized attachment when both were present, as compared with the absence of both. The same group (Bakermans-Kranenburg et al. 2008) reported even more recently that the cortisol-reducing effect in 1 to 3-year-old children of a video-feedback intervention to promote positive parenting and sensitive discipline was only apparent in the presence of the 7-repeat polymorphism. Schmidt et al., (2007) studied behavioral problems in 7-year-olds and found that children with a long version of the 7-repeat allele of the DRD4 gene had more internalizing and externalizing behavior problems, as did children with one or two copies of the short allele of the serotonin transporter gene. The long version of the serotonin transporter gene protected against the influence of the long version of the 7-repeat allele of the DRD4 dopamine gene. In a public-speaking test, Gilissen

et al. (2008) found that the long version of the serotonin transporter gene in interaction with attachment security had a protective effect against stress in 7-year-olds.

As for twin studies, Kendler (1996) administered the Parental Bonding Instrument to adult female twins and asked them to report on the warmth, protectiveness and authoritarianism they had themselves received from their parents, their twin sister had received from the parents and they themselves provided to their own children. The parents of the adult female twins were also administered the PBI. The study revealed that warmth was partly heritable, to an extent that differed between the groups. Protectiveness and authoritarianism were largely the result of environmental factors. Finkel, Wille & Matheny (1998) used a modified Strange Situation to measure attachment in twins. Concordance for monozygotic twins was considerably higher then for dizygotic twins (68% vs. 39%). The same group (Finkel & Matheny 2000) reported that 25% of the variability in a sample of 207 monozygotic and dizygotic twins was attributable to genetic factors, and the rest to non-shared environmental effects. Van IJzendoorn et al. (2000) investigated Strange Situation attachment concordance in 138 sibling pairs from different countries. They found a 62% concordance when looking at the secure vs. insecure difference. As this concordance is comparable with the concordance previously found in monozygotic twins, they concluded that genes do not play a major role.

O'Connor & Croft (2001) assessed 110 pairs of twins in the Strange Situation and found a concordance between mono- and dizygotic twins of 70% and 64% respectively, suggesting little influence of genetic factors. Bokhorst et al. (2003), in a sample of 157 mono- and dizygotic twins, found that 52% of the variance in secure vs. insecure attachment in the Strange Situation was the result of shared environmental effects and 48% of non-shared environmental effects and measurement errors, suggesting that genes did not have any influence. This study on attachment to the mother was followed by a study in which mothers of 56 mono- and dizygotic twins rated attachment behavior of their children towards the fathers with an Attachment Q-sort (Bakermans-Kranenburg et al. 2004b). Genetic modeling showed that attachment was largely explained by shared environmental (59%) and unique environmental (41%) factors. The most recent twin study (Fearon et al. 2006), in which maternal sensitivity was also measured, also showed little evidence of heritability. Torgersen et al. (2007) used the Adult Attachment

Interview to study same-sex mono- and dizygotic adult twins. In contrast to the early childhood studies, they found that heredity played a significant role.
As to the correlation between the infant's attachment to the mother and to the father, van IJzendoorn & de Wolff (1997) performed a meta-analysis of 14 studies with 950 families. They found a limited correlation phi of .17, indicating little influence of genetic factors.
In conclusion, it can be said that there is some evidence that the risk of disorganized attachment in children is partly influenced by genetic factors. Other studies, with the exception of one study in adults, have yielded little evidence of heritability, confirming the dyadic nature of the attachment concept.

3) *Cognitive representations of attachment experiences should determine to a certain extent the attachment behavior (i.e. empathic reactions like sensitivity/responsiveness) of the care-giver, which in turn should influence the attachment behavior of the child. This means that there should be evidence of inter-generational transmission of attachment classification, preferably mediated by the empathic behavior of the care-giver.*

In a meta-analysis on this subject, van IJzendoorn (1995) analyzed 18 studies with 854 mother-infant pairs and showed that the Adult Attachment Interview classification of maternal attachment predicted the Strange Situation classification of the infant rather well (effect size 1.06). In the same article, an effect size of .72 was found as a result of a meta-analysis of 10 studies (N=389) investigating the relationship between AAI security and maternal sensitivity, see also Biringen et al. (2000) for a comparable result. In a meta-analyis of 21 studies in nonclinical samples using observational measures of sensitivity of the care-giver and the Strange Situation classification of the infant, de Wolff & van IJzendoorn (1997) found an effect size of .24, which is considered moderate. In later studies, maternal sensitivity was described as being weakly (True et al. 2001; Atkinson et al. 2005; Fuertes et al. 2006) or moderate to strongly related to infant security (NICHD Early Child Care Research Network 2001; Belsky & Fearon 2002; Campbell et al. 2004 - both drawing from the NICHD study; Tarabulsy et al. 2005; Tomlinson et al. 2005; Britton et al. 2006; Fuertes et al. 2006; Veríssimo & Salvaterra 2006). A meta-analysis by Bakermans-Kranenburg et al. (2003) also showed that sensitivity can

be changed by (preferably short-term) behavioral interventions and that interventions that were more effective in enhancing sensitivity in the mother were more effective in changing attachment insecurity in the child.

The relationship between cognitive/mental state factors in the care-giver as measured with the AAI and infant attachment security is rather strong (in both mothers and fathers, Steele et al. 1996). The relationship between AAI and care-giver sensitivity and the relationship between care-giver sensitivity and infant attachment status is also rather strong. This does not mean however, that sensitivity mediates the relationship between the care-giver's attachment and the infant's attachment. The studies measuring all three did not find this mediation, possibly leaving a role as moderator (Atkinson et al. 2005; Bailey et al. 2007). The situation in the specific problem of unresolved maternal attachment/frightening behavior/disorganized attachment in the children is practically the same as reviewed by Madigan et al. (2006). There is an association between unresolved maternal attachment and anomalous parenting styles (.26), between parenting and disorganized attachment in the children (.34) and less of an association between maternal and infant attachment (.21). There is no clear evidence of the mediating role of maternal behavior. The question as to how the cognitive attachment status of the care-giver is transmitted to the behavior of the child remains unanswered ("transmission gap", van IJzendoorn 1995). Alternative ways of predicting inter-generational transmission of attachment are thus being pursued, for instance by Fonagy et al. (1993). They found that the capacity to reflect upon the mental states of self and others correlated positively with infant attachment security. Among others, some recent studies (Grienenberger et al. 2005; Slade et al. 2005) confirm this finding. Yet reflective functioning is essentially a cognitive factor that strongly resembles some aspects of the coding of the Adult Attachment Interview. Even if reflective functioning in the mother predicts infant security better then the AAI, this does not solve the problem of the absence of a behavioral mediating variable.

A second alternative is to acknowledge that contextual influences are of relevance and can act as intervening variables. Atkinson et al., (2000) conducted a meta-analysis of maternal health correlates and found that social-marital support (r=.14), stress (r=.19) and depression (r=.18) were each correlated with infant attachment status.

A third alternative is proposed by Dickerson Peck (2003), who suggests that behavioral micro-analysis could trap the subtle interactions in a better way than existing measures and that the suggested modest role of maternal sensitivity is in fact a measurement problem. A study by McElwain & Booth-Laforce (2006) showing that greater sensitivity to distress, but not to non-distress, predicted that attachment security supports this. A comparable view, although from an entirely different theoretical perspective, is held by Kassow & Dunst (2004). They performed a meta-analysis in which they only looked at responsiveness in the parent that was contingent on infant behavior. The review was based on Gewirtz' view from a behaviorist perspective (Gewirtz & Peláez-Nogueras 1991) that attachment behavior is the pure result of operant conditioning. Kassow & Dunst found an effect size of .59, considerably higher than the .24 of de Wolff & van IJzendoorn. They also found that the earlier in the infant's life the responsiveness was measured, the higher the effect size. This finding suggests that a timely, well-defined and limited, possibly more micro-analytic measurement of sensitivity/responsiveness, could bridge the transmission gap and reinstate maternal sensitivity as a mediator between maternal and infant attachment.

4) *Because secure attachment promotes cognitions of self-efficacy and confidence in seeking proximity and support as a way of regulating distress, psychopathology is expected to be more prevalent in people with insecure attachment than in people with secure attachment.*

Van IJzendoorn et al., (1999), in a meta-analysis, found an effect size of .29 of disorganized attachment on problematic behavior in children. Other reviewers (Green & Goldwyn 2002; Zeanah et al. 2003) also stress the dominant role of disorganized attachment in psychopathology, especially in children. There has not been much research into the role of disorganized attachment in adult psychopathology. In paragraph 1.2.B of this introduction, a review of the relationship between attachment and psychopathology is presented. The majority of studies (both prospective and cross-sectional) show that attachment insecurity is a risk factor for psychopathology.

5) *The attachment behavioral system, evolved under the influence of natural selection, organizes interactions that lead to the development of cognitive working models on the basis of real life experiences. These experiences will have the greatest effect during the period of most rapid neurobiological development, leading to a relative stability of the attachment style after the first few years of life. Yet, because of the adaptive function of the working models, a consistently different attachment context can lead to changes in attachment style.*

In their adoption study mentioned under hypothesis (3), Veríssimo & Salvaterra (2006) found that maternal secure base scripts had a greater influence on the secure base behavior in children than the age of adoption or the age of measurement. This means that attachment experiences in the first years of life were "overruled" by later experiences. Waters et al. (2000) found that 71% of 50 white, middle-class subjects kept the same secure vs. insecure classification from the Strange Situation in infancy to an Adult Attachment Interview at 21 years of age. A change in classification took place in 44% of children whose mothers reported serious life-events and in 22% of those without such events. Hamilton (2000) also reported a 77% secure vs. insecure stability in 30 subjects and a significant influence of life events on changes in typology. Weinfield et al. (2004) did not find continuity of attachment classification in a sample of 125 high-risk subjects. There was again evidence, however, for the influence of life events on change in typology. This suggests that both in low-risk and high-risk samples, stability of attachment classification is dependent on the amount of intervening life events and that this amount is higher in high-risk samples, leading to a dissolving stability. Fraley & Brumbaugh (2004) performed a meta-analysis and found a correlation from age 1 to age 4 of .35, to age 6 of .67 and to age 19 of .27. They conclude that attachment status at age 1 is not a strong predictor of later attachment, but that it is enduring, predicting attachment that is about as precise over a span of 3 as over a span of 18 years. Fish (2004) and Moss et al. (2005) subsequently reported low to moderate stability over a 2-2 ½-year period in pre-school children, but with clear external factors in children who changed from secure to insecure attachment and vice-versa or who changed from secure to disorganized attachment.

In adulthood, attachment is more stable than during childhood. Klohnen & Bera (1998) found a correlation from age 27 to age 43 of

.58, and from age 27 to age 52 of .55. Fraley & Brumbaugh (2004) found a correlation of .54 as a result of a meta-analysis, but hardly any study had a test rest period of longer than 2 years and most were much shorter. In conclusion, the hypothesis as to stability of attachment representation is to a large extent confirmed. Although the influence of environmental variables is larger than expected, working models seem rather stable after the first year, in the absence of attachment trauma. In adulthood, stability is greater and environmental influences are smaller.

1.2.B General conclusion

The 5 hypotheses mentioned above are generally supported by research data, where available. The influence of genetic factors on attachment patterns is probably somewhat stronger than expected, but still rather limited in comparison with other findings in psychology and psychiatry. The stability of the attachment classification through time is less than expected, on the one hand more or less disconfirming Bowlby's idea about the sensitive period, but on the other hand, confirming his emphasis on real-life experiences in the construction of working models. What is still substantially lacking, despite recent advances, is a link between behavioral/psychological data and biology. The formal use of attachment theory in the clinical practice of psychology and psychiatry is limited, certainly in the light of the dominance with regard to scientific evidence that attachment theory has over other theories in developmental psychology. However, attachment research has, until recently, neglected the neurobiological aspects. This is a puzzling phenomenon, given the emphasis Bowlby gave to the evolutionary – and thus biological– development of the attachment behavioral system. Meanwhile, clinical psychology and psychiatry have oriented themselves excessively in recent decades towards the biological aspects. This discrepancy has contributed to a lack of penetration of attachment theory in everyday clinical practice that is extraordinary, given the absence of evidence-based alternatives.

I.3 Clinical relevance of attachment theory: attachment and psychopathology

I.3.A Measurement of attachment

The Strange Situation Test (Ainsworth 1978) is a laboratory experimental procedure, in which a child between 12 and 24 months is brought into a new environment with the mother or another attachment figure. The child is invited to play and then a stranger enters the room and starts to play with the child. The mother leaves the room for a short period and then re-enters. In the next phase the stranger and the mother leave and the child is left alone, then the stranger re-enters and finally the child is reunited with the mother. The phases each last just a few minutes. Children are classified on the basis of the different forms of behavior of the child, such as the exploration of the strange room in the presence of the mother, the reaction to the entrance of the stranger, behavior after the departure of the mother and the reaction to being reunited. They can be classified *securely* attached, in which case the children are able to explore the room in the presence of the mother, are distressed by her leaving, seek proximity when she re-enters and are comforted by her in reasonable time, after which they are able to resume their play. Children who are *avoidantly* attached do not seem to care very much if the mother is present, do not turn to her for comfort after reunion and even seem to avoid her. Physiological measures, however, show that these children are stressed by the procedure and thus, in the interaction with the parent, seem to have learned to suppress attachment behavior. The *ambivalently* attached child, on the one hand, gets very distressed by the departure of the mother, clings to her upon return, but on the other hand, shows signs of rejection, for instance refusing to be put on the mother's lap. They are difficult to console and they do not return to play, but often remain seated at the feet of their mother, crying or angry. The *disorganized* category (discovered after the original 3 category coding system did not fit a number of children who lacked a specific behavioral strategy) shows contradictory or odd behavior, like walking towards the mother while gazing away from her, or suddenly stopping and remaining motionless for a number of seconds. There are indications of fear of the mother. In short: *secure* children turn to the mother for support and know how to use it, *avoidant* children pretend to be independent of the mother's support, *ambivalent* children excessively demand support because they do not expect an adequate and consistent response and *disorganized* children are at a loss as to what to do.

An instrument often used during home observations is the Attachment Q-sort (Waters & Deane 1985). Secure base behavior of children of one to five years old in interaction with the mother is observed and subsequently 90 cards containing statements about the behavior are sorted by the observer. Meta-analysis (van IJzendoorn et al. 2004) showed that the Attachment Q-sort adequately predicts both Strange Situation classifications of the child and sensitivity measures of the mother.
Measurement of maternal sensitivity is often still done with the original Ainsworth system (Ainsworth et al. 1978) or with a Maternal Behavior Q set (Pederson & Moran 1995).

The Adult Attachment Interview (George, Kaplan & Main 1985) is an interview approximately one hour long about attachment-related events during childhood, reflections upon the nature of the attachment relationships with the parents and the effects of them on the present personality. The interview is recorded, typed out verbatim and then coded from the transcript by an experienced rater who was not present during the interview. Respondents are classified according to a scoring system devised by Main & Goldwyn (1993) or with a Q-sort technique (Kobak 1990). The scoring is based largely on structural aspects of the transcript, i.e. the presence of clear and coherent memories, the presence of contradictory emotions, presence and relevancy of details etc. Respondents can be classified as autonomous, dismissing, preoccupied or unresolved. *Autonomous* subjects have a balanced and empathic view of themselves and their parents, they acknowledge the limitations and the positive sides of their parents and they have a coherent discourse about their childhood. *Dismissing* subjects tend to be vague, although often positive, about their childhood and provide little detail. They tend to minimize the importance of early relationships for later development and overvalue independence. They can get irritated by the type of questioning. *Preoccupied* subjects are still emotionally very involved in the events of their childhood and are not able to get beyond details and get an overview or more balanced picture. They are still rather concerned about pleasing their parents and/or are still angry with them. The *unresolved* category has not succeeded in passing the mourning stage of the loss of a significant other or in integrating events of traumatization. They are cognitively confused on these subjects, may

not grasp the reality of them and may still have feelings of guilt or responsibility.
Hesse (1999) provided an extensive review of the validity of the Adult Attachment Interview and of the research that has been done with it.

The Parental Bonding Instrument (PBI, Parker et al. 1979) asks adults to remember their parents during the first 16 years of life and consists of 25 questions for each parent. The original meaning was to create two factors: 12 items referring to parental care and 13 items referring to parental over-protection. Later (Kendler 1996), a three-factor structure seemed a better match with the data, with the third factor being authoritarianism. This result was replicated in the National Comorbidity Survey (Cox et al. 2000), where only 8 items yielded the three-factor structure. Additional confirmation of the three-factor structure was found in a recent European study (Heider et al. 2005). The three-factor analysis is promising, as Lizardi & Klein (2002) found that distinction between sub-types of depression became possible when the two-factor analysis was replaced by the three-factor one. Almost all studies to date, however, have been analyzed using only care and over-protection. The Parental Bonding Instrument is a valid instrument (Parker 1989) that measures what it claims, the scores being independent of other factors (e.g. depression, Nitta et al. 2008). Yet the PBI is a self-report measure of parenting as perceived by the adult and does not convey information about the attachment status of the subject.

Hazan & Shaver (1987) developed a self-report measure of the original three types of attachment for adults in romantic relationships. This was the starting point of a cascade of developments in the self-report measurement of attachment (see Crowell et al. 1999 for a review). The fourth category, disorganized attachment, was added, continuous scales were developed and after a factor analysis of all existing dimensional attachment measures in English (Brennan et al.1998), it was concluded that they all fit into a two-dimensional space. One dimension is anxious attachment, the other is avoidance of attachment. The first deals with appraisal of attachment-related threats, such as (possibly) the absence of the attachment figure. The second deals with the preferred behavioral strategy, i.e. turning to the attachment figure or withdrawing and handling things alone (Fraley & Shaver 2000a). People who are low on anxiety and low on avoidance can be placed in the secure category, people high on anxiety and low

on avoidance are preoccupied, people high on anxiety and high on avoidance are fearful-avoidant and those low on anxiety and high on avoidance are dismissive-avoidant. The instrument that was the result of the factor analysis by Brennan et al., is the Experiences in Close Relationships Scale, a revised edition (Fraley et al. 2000b) of which now exists.
There has been considerable debate about the usefulness of self-report vs. interview measures (Crowell et al. 1999; Shaver & Mikulincer 2002; 2004). Self-report measures would not be able to tap into unconscious processes and thus would miss dismissive strategies, i.e. the unconscious exclusion of negative emotions concerning attachment. On the other hand, categorical interview measures would be less sensitive than dimensional self-report and would be unsuitable for measuring the different attachment relationships that individuals can have etc. From a psychopathological point of view, one can say that the absence of the disorganized category in the two-dimensional self-report measures of adults is bothersome, since one sees that psychopathology in children is most prevalent in those with disorganized attachment.

1.3.B Clinical studies

This review is exclusively focused on correlations between attachment variables and the presence of psychopathology. Studies on the relationship between attachment and behaviors, beliefs or emotions that do not refer to a specific diagnosis were excluded, with the exception of the categories of internalizing and externalizing behavior in children. This was done because of the general opinion in child psychiatry that these categories are an acceptable path to document psychopathology.
For practical reasons, studies on the relationship between parental attachment variables and the presence of psychopathology in their children are not included, nor are studies concerning the relationship between attachment variables in patients and the course of the illness. Research into attachment correlates of subtypes of disorders is also excluded.
In this review, interview and observation measures precede self-report measures. Research on psychopathology in adolescents and adults is presented together.

1.3.B.1 Psychopathology in children.

A number of reviews concerning the relationship between attachment and psychopathology in children have been published in past years. Van IJzendoorn et al. (1999) performed a meta-analyis on the sequelae of disorganized attachment and reported an effect size of .29 on the occurrence of externalizing problem behavior. A landmark study is the Minnesota longitudinal study (Carlson 1998), which followed 157 high-risk children from birth until 19 years of age. Disorganized attachment in childhood as measured in the Strange Situation Test correlated with behavior problems throughout the school period and psychopathology and dissociation in adolescence. A review by Greenberg in the same year as that of van IJzendoorn (1999) was in agreement with van IJzendoorn that disorganized attachment is a risk factor for externalizing problem behavior but adds that to a lesser extent, this is also true for avoidant attachment. Greenberg also concluded that, to his surprise at the time, there were no studies on the relationship between attachment and depression or anxiety in children. Given the role that Bowlby presumed for both depression and anxiety in the development of insecure attachment, this is indeed strange. Much attention has been devoted to the influence of maternal depression on attachment security of their children (e.g. Campbell et al. 2004) but this is beyond the scope of this introduction. Green & Goldwyn (2002) also focused on disorganized attachment in their review of new findings in attachment research and developmental psychology. They concluded that the shift from secure vs. insecure to organized vs. disorganized attachment has opened a new phase in research. But they also concluded that disorganized attachment is, as yet, a broad concept that does not seem to represent more than a non-specific vulnerability with multiple causes. Zeanah et al. (2003) reviewed the relation between attachment and psychopathology with a special emphasis on the DSM-IV reactive attachment disorder. They reiterated the conclusion that disorganized attachment is most clearly related to psychopathology, citing van IJzendoorn and Bakermans-Kranenburg (2003), in that it could be called a clinical syndrome as such. Zeanah et al. state that the relationship between attachment organization and the clinical syndrome of reactive attachment disorder is not quite clear. Some children with RAD do not show any type of attachment at all, while others – although RAD is the result of extreme neglect – can be classified as secure. Also, the inhibited and the disinhibited subtype of

reactive attachment disorder are so different that it is unlikely that they are the consequence of the same attachment strategy.

In recent years, the findings of the reviews above have been confirmed. Munson et al. (2001) showed that both avoidant and disorganized attachment in the Strange Situation in pre-schoolers predicted externalizing behavior problems when they were 9 years old, as did maternal depression. McCartney et al. (2004), reporting on 1364 children from the NICHD study of Early Child Care, found that insecure attachment in the Strange Situation at 36 month of age was a predictor of mother and care-giver ratings of internalizing problems for boys and girls and externalizing problems for boys at age three. Maternal depression also predicted both internalizing and externalizing problems.
Edwards et al. (2006) investigated children of alcoholic fathers and concluded that secure attachment to the mother at 12 months reduced the rate of internalizing and externalizing behavior at 18-36 months. Moss et al. (2006) measured attachment in 6-year-olds and externalizing behavior when they were 8. Both ambivalent and disorganized attachment predicted externalizing behavior. Only disorganized attachment predicted internalizing problems. Smeekens et al. (2007) found that disorganized attachment at 15 months predicted externalizing behavior at 5 years of age, together with other parent and child related factors. Madigan et al. (2007) measured attachment in mothers with the Adult Attachment Interview t 6 months post partum, infant mother attachment in the Strange Situation at 12 months and externalizing problems at 24 months. Unresolved loss in the mothers and disorganized attachment in the Strange Situation predicted later externalizing behavior problems. Muris and Maas (2004) investigated two groups of children with below-average intellectual abilities. One group was institutionalized, the other was not. Attachment was measured with the Attachment Questionnaire for Children (Muris et al. 2001). In both groups, insecurely attached children showed more psychopathology, while the institutionalized group was more often insecurely attached than the non-institutionalized.

Since Greenberg's (1999) surprise about the lack of studies on the relation between attachment and anxiety/depression, several studies have been carried out on the subject.

Graham and Easterbrooks (2000) examined the influence of attachment security (in an adaptation of the Strange Situation test for older children), maternal depression and economic status on depressive symptomatology in 85 children aged 7-9. These three factors accounted for 47% of the variation, while secure attachment served as a buffer. Muris et al. (2000) used a simplified version of the single item Hazan & Shaver (1987) measured in 12-year-olds and found that insecure children had higher levels of anxiety and depression.

Abela et al. (2005) studied 140 children of parents with a history of major depression. Insecure attachment, in interaction with excessive seeking of reassurance, predicted both present and past occurrence of depressive symptoms in the children. Shamir-Essakow et al. (2005) investigated 3 to 4-year-old at-risk children in the Strange Situation and performed a DSM-IV classification of anxiety disorder. Bar-Haim et al. (2007) performed the Strange Situation test at 12 months and had children and mothers completely screened for anxiety symptoms at age 11. Compared with the securely attached children, ambivalently attached children had higher levels of school and social phobia, although this did not reach the clinical level. Insecure attachment and behavioral inhibition predicted anxiety disorder, even after controlling for maternal anxiety. Stein et al. (2000) compared children with major depression with high-risk children (without actual depression but with depression in the family) and low-risk controls. They used a child version of the PBI. Children with depression differed from the controls as they reported maternal over-protection. Maternal depression negatively influenced PBI scores in depressed and non-depressed children.

In pediatric eating disorders, anorexic toddlers showed more insecure attachment in the Strange Situation than the controls (Chatoor et al. 1998), as did their mothers (Chatoor et al. 2000). Meesters et al. (2007) found an association between eating problems in 10-16 year-olds and parental control and maternal rejection as measured by the EMBU-C, a child version of the EMBU. The EMBU (Egna Minnen Betraffande Uppfostran, Swedish for “my memories of upbringing”, Perris et al. 1980) is a three-factor instrument, designed to measure memories of parents’ behavior in adults. The three factors correspond to the three-factor PBI. In the same study, insecure attachment to the mother according to the Relationship Questionnaire for Children (an adaptation of the Bartholomew and Horowitz 1991 four-category instrument) was associated with eating problems.

Finally, in a meta-analytical review on autism and related disorders, Rutgers et al. (2004) concluded that insecure attachment is more often present in autistic children then in controls. Two factors explained this relationship: the more severe the autistic symptomatology, the more insecure was attachment and the presence of mental retardation seemed to explain the major part of attachment insecurity in autism. The same group (Naber et al. 2006) compared toddlers with autism with toddlers having other developmental disorders (pervasive developmental disorder, mental retardation and language development disorder) and normal controls. In contrast with most studies in the review, disorganized attachment was included in the Strange Situation scoring. The clinical group was more often insecurely and disorganized attached, with no statistical difference between these groups. The severity of autistic symptoms was correlated to attachment insecurity and developmental level was negatively correlated to disorganized attachment. The same group (Rutgers et al. 2007) subsequently used a short attachment screening questionnaire in the same clinical groups and a control group and found that children with autistic spectrum disorder were less securely attached.

The outcomes of the different reviews and the results of later publications lead to the **conclusion** that insecure attachment is a risk factor for psychopathology in children. More specifically, avoidant attachment increases the risk of externalizing behavior problems, while disorganized attachment greatly increases the risk for childhood psychopathology in general, but again especially for externalizing behavior disorders.

1.3.B.2 Psychopathology in adolescents and adults

1.3.B.2.a Studies on psychopathology in general

Dozier (1990) used a Q-sort rating of the Adult Attachment Interview (AAI) to get scores on a Security/Anxiety scale and an Avoidance/Preoccupation scale. She compared a group of in-patients with psychotic and affective disorders with a group of college students and a group of adults that had participated in a study of marital functioning. Psychiatric patients were significantly more anxious than the other two groups. There was no difference on the Avoidance/Preoccupation dimension. Allen et al. (1996) re-interviewed a group of 66 people at the age of 25 with the AAI, who

had been psychiatrically hospitalized at the age of 14. They compared them with 76 people from the same socio-demographic group. Almost all ex-patients were insecurely attached, with the disorganized type prevailing. Insecure attachment was linked to self-reported hard drug abuse and criminality.
Fonagy et al. (1996) compared 82 non-psychotic inpatients with 85 matched controls with the AAI. Patients, who were more likely to be preoccupied or unresolved with regard to trauma or loss. Rosenstein & Horowitz (1996) examined the relationship between attachment classification and psychopathology and personality disorders in a group of 60 adolescent psychiatric in-patients. Attachment status in adolescents as measured with the AAI concurred with maternal attachment status and was almost exclusively insecure. Wallis & Steele (2001), using the AAI, found an over-representation of insecure attachment, related in particular to unresolved trauma or loss in a group of 39 adolescent psychiatric in-patients. Ward et al. (2006) compared 30 women with a diagnosis in the Structured Clinical Interview for DSM-III-R with 30 women without such a diagnosis. Insecure attachment correlated to the presence of a diagnosis, while 32% of women with secure attachment, 63% of women with dismissing attachment, 100% of women with pre-occupied attachment and 65% of women with unresolved attachment had a diagnosis. In women with unresolved problems, an underlying classification of secure attachment correlated to low psychopathology, while an underlying classification of anxious attachment correlated to high psychopathology.

West and colleagues (1993) used a three-factor instrument (feared loss of the attachment figure, proximity seeking, and separation protest, West & Sheldon 1988) to differentiate between 110 psychiatric outpatients and 136 respondents in a community survey. All three factors were significantly involved in the distinction, feared loss of the attachment figure dominating in particular. In a non-clinical sample of late adolescent women, the Revised Adult Attachment Scale (Collins & Read 1990, designed to categorize subjects as securely, anxious or avoidant attached) and the Inventory of Parent and Peer Attachment (Armsden & Greenberg 1987, measuring degree of trust, quality of communication, and degree of anger and alienation) predicted various types of DSM-II-R Axis I and Axis II disorders 12 months later (Burge et al. 1997). There was no specific relationship between sub-scales or categories and type of disorder. Mickelson et al. (1997)

reported on the results of the National Comorbidity Survey association between a Hazan & Shaver-like instrument and life prevalence of DSM-III-R psychiatric disorders. Avoidant and anxious attachment were correlated to all types of psychopathology. The only exception was that anxious attachment was not related to alcohol and drug abuse. Muller & Lemieux (2000a) carried out research in a group of adults who were physically or sexually abused as children and investigated whether attachment factors were correlated to the presence of psychopathology. They used the Relationships Scales Questionnaire (Griffin & Bartholomew 1994) and a number of other measures of risk and protective factors. The RSQ can be used to assess the two dimensions of the model of the self and model of the other. The scale for a negative view of the self correlated the most to psychopathology.
Parental variables alone contributed significantly to externalizing pathology. Brown and Wright (2003) compared a clinical group of adolescents with a non-clinical group, using a modified Separation Anxiety Test. Preoccupied attached adolescents scored higher on symptoms and inter-personal problems, while secure and dismissive styles did not. Marazziti and colleagues (2007) used the scale of Experiences in Close Relationships to compare a group of patients with mood and anxiety disorders with normal controls. In the controls, secure attachment prevailed, while in the patients, the preoccupied style was the most frequent, with anxiety and avoidance scores also significantly higher. There were no significant differences between diagnostic groups.

Mackinnon et al. (1989) did not find an association between scores on the Parental Bonding Instrument and psychopathology in a sample of 386 people from the general population of Australia. Enns et al. (2002) reported from the US National Comorbidity Survey that lack of parental care, as measured by an 8-item version of the Parental Bonding Instrument, increased the risk for psychopathology in the adult population in a non-specific way. The effect was small, accounting for only 1-5% of variance. High scores on paternal over-control and authoritarianism, on the other hand, reduced the risk of substance abuse and anti-social personality disorder. In **conclusion**, insecure attachment can be considered a risk-factor for psychopathology in general, with the preoccupied and unresolved sub-types outweighing the dismissive sub-type.

1.3.B.2.b Schizophrenia and other psychotic disorders

Zechowski et al. (2004) used a Polish version of the Inventory of Parent and Peer Attachment in a group of 35 schizophrenic adolescents. There were no differences with healthy controls in parent attachment. A Portuguese translation of the Bell Object Relations Inventory (Bell et al. 1986) showed that Brazilian schizophrenic patients were more insecurely attached than healthy controls (Bell & Bruscato 2002).

Ponizovsky et al. (2007) compared 30 schizophrenic patients with 30 normal controls and found that self-reported insecure attachment, especially avoidant attachment, was correlated to schizophrenia and a worse course of the illness.

Parker et al. (1982) matched 78 schizophrenic patients with controls from general practice. Schizophrenics scored both their parents lower on care on the Parental Bonding Instrument (PBI), and their fathers higher on control. Scoring one or both parents in the low care/over-protection quadrant predicted an earlier age of first admission and relapse. In a later study (Parker et al., 1988), only the paternal scores of low care and over-protection ("affectionless control") differentiated schizophrenic patients from controls and predicted relapse, but earlier age of first admission was not confirmed. Byrne et al., (1990) compared 14 schizophrenic patients with a general population sample. They found that schizophrenics scored their fathers lower on care. Onsted et al. (1993) administered the PBI to a group of 31 monozygotic and dizygotic twins that were discordant for schizophrenia. The patients reported less care and more over-protection in both parents than their healthy twins, paternal over-protection being the most distinctive factor. Helgeland & Torgersen (1997) only measured maternal perceived parenting with the PBI and also found less care and more over-protection in schizophrenic patients than in controls. Willinger et al. (2002) found the same result, but when they controlled for premorbid personality traits, only maternal overprotection remained significant. Skagerlind et al. (1996) used the EMBU to compare 57 schizophrenic patients with healthy controls. Patients rated both their parents higher on rejection and their mothers lower on emotional warmth. EMBU scores did not predict clinical course.

To **conclude**, the lack of research (especially with interview measures) into such a major disorder as schizophrenia is puzzling. The research that has been done, predominantly with the PBI, shows a

rather consistent correlation to perceived lack of warmth and of over-protection/control from the parents, with the mother and the father alternating or both involved.

1.3.B.2.c Depression

Adam et al. (1996) interviewed suicidal adolescents and clinical controls. Pre-occupied and disorganized attachment (according to the AAI) were over-represented in the suicidal group.
Insecure attachment correlated to the severity of depression, partly mediated by the dysfunctional attitudes. Bifulco et al. (2002a) used the Attachment Style Interview, designed to classify attachment into 5 different categories, with dismissive attachment being divided into an angry-dismissive and a withdrawn sub-category. The instrument is also meant to assess the degree to which the insecure attachment styles deviate from normality. With the ASI, Bifulco and colleagues found that insecure attachment styles were related to depression in a 12-month period and partially prospective. Only the withdrawn sub-category did not contribute to depression (Bifulco et al. 2002b). In a later study (Bifulco et al. 2006), the same group investigated 154 high-risk women. Fearful and angry-dismissive attachment styles partially mediated the relationship between childhood adversity and adult psychopathology. Moderate to marked insecure attachment predicted major depression. The fearful style was predominantly responsible for the association. A Portuguese translation of the ASI was used in a comparison of pregnant teenagers and adult women (Figuiredo et al. 2006). Enmeshed, fearful and angry-dismissive styles predicted depression in the teenagers, with enmeshed style being the most prominent.

Armsden et al. (1990) measured parent and peer attachment with the IPPA in a group of clinically depressed early adolescents and compared them with non-depressed psychiatric controls, non-psychiatric controls and a group in which the depression was resolved. Parent attachment was significantly less secure in the depressed groups than in the three other groups. Among the psychiatric groups, severity of depression was negatively correlated to security of attachment. Roberts et al. (1996) administered a variation on Hazan & Shaver's three-factor test to 144 undergraduate students and measured depressive symptoms. Secure attachment was negatively correlated to depression, while ambivalent and avoidant styles were positively

correlated. Dysfunctional attitudes and low self-esteem mediated these correlations. West et al. (1998), using the Reciprocal Attachment Questionnaire, found that women who scored in the clinical range of the Centre for Epidemiological Studies Depression Scale (CES-D) were significantly more often anxiously attached than the women who scored in the normal range. DiFilippo and Overholser (2000) examined depression and suicidal ideation in 59 adolescent in-patients. Through a series of hierarchical regression analyses, they found that attachment to mothers, as measured with the IPPA, was negatively correlated to severity of depression, which in turn accounted for suicidal ideation. Attachment to peers accounted for both depressive symptoms and suicidal ideation, but only in girls. Hammen et al. (1995) followed 129 young women for a year and investigated whether RAAS (Revised Adult Attachment Scale, Collins & Read 1990) scores predicted onset of depressive symptoms as measured by the SCID (Structured Clinical Interview for DSM-III-R). The RAAS has three sub-scale scores (Closeness, Dependency, Anxiety) and depression was predicted marginally by the Closeness score, but more strongly by the Dependency score, especially in interaction with inter-personal life events. Reinecke and Rogers (2001) also used the RAAS in fifty-four depressed women, together with scales for dysfunctional attitudes and depression. Anxiety correlated positively to depression, Closeness and Dependency negatively. The influence of attachment on depression was partly mediated by dysfunctional attitudes.

Shaver et al. (2005) examined the relationship between excessive reassurance-seeking and depression in young couples and found that the relationship was mediated through anxious attachment, not, however, through avoidant attachment, as measured with the Experiences in Close Relationships Scale.

McMahon et al. (2005) carried out a prospective study on pregnant women and found that depression at 12 months post-partum was predicted by low maternal care during childhood, marital dissatisfaction and anxious attachment. Anxious attachment mediated the influence of low maternal care. Monk et al. (2008) also studied the relationship between attachment style and depression in pregnant women using the Relationship Questionnaire. They found that insecure attachment styles were positively correlated during pregnancy to depressive symptoms, as measured with the CES-D, the fearful style more than the preoccupied and the dismissive style. Secure attachment was negatively correlated to depressive symptoms.

Surprisingly, fearful attachment was negatively correlated to depressive symptoms at 4 months post-partum, as was secure attachment. Other insecure attachment styles were not correlated to post-partum depression. Hankin et al. (2005) carried out three prospective studies in university students on the relationship between attachment type and depressive and anxiety symptoms. In two studies the RAAS was used, in the third, the Adult Attachment Questionnaire, a 10-item version of Hazan & Shaver's original instrument. Avoidant and anxious attachment both predicted depressive symptoms. Interpersonal and cognitive variables mediated these relationships. Zech et al. (2006) found that anxious attachment in patients visiting their general practitioner, as measured by a renewed factor orientation of the Adult Attachment Scale questions, was positively correlated to the depressive affect, while avoidant attachment was negatively correlated to it. Runkewitz et al. (2006) also carried out a study in a general practice, using the Hospital Anxiety and Depression Scale (HADS) and the Bielefeld Questionnaire on Partner Expectations, a German instrument. Insecure attachment predicted clinically relevant depression scores, ambivalent attachment somewhat higher than avoidant attachment.
Meredith et al. (2007) studied depression in pain patients and reported that the Attachment Style Questionnaire factor 'relationship anxiety' was positively correlated to pre- and post-treatment depression scores, while comfort was negatively correlated to closeness. Comfort to closeness was the best predictor of treatment success, better than depression or pain scores.

In table 1, results of studies linking the Parental Bonding Instrument to depression are summarized. Depression is clearly associated with perceived low parental care, while the number of studies reporting low maternal care is somewhat greater than those reporting low paternal care. In nearly half of the studies, there is evidence of association with over-protection, again more often maternal than paternal. Studies in non-clinical groups exclusively report positive associations between depression and low parental care, while there is no difference with clinical groups in the number of studies that report associations with over-protection. The difference between clinical and non-clinical groups can be tentatively explained by the use of more sensitive dimensional instruments in the latter instead of categorical/diagnostic depression measures. The fact that this does not alter the number of studies reporting an association with over-protection implies that this

association is weaker than the association with low parental care. Narita et al. (2000), Lizardi et al. (2002) and Martin et al. (2004) used both the two-factor and the three-factor analysis of the PBI. Narita et al. found evidence for the contribution of over-protection in the three-factor analysis that was not present in the two-factor analysis. Lizardi et al., however, lost a significant relationship with maternal protectiveness (a term used to describe one aspect of over-protection from the two-factor analysis) when switching to the three-factor analysis. In the three-factor analysis, an association with maternal 'authoritarianism' appeared, suggesting that this is the pathogenic aspect of over-protection in the two-factor analysis. The result of the study by Martin et al. (2004) confirmed this, showing evidence for a two-factor structure of over-protection, with 'intrusiveness' contributing to the presence of depressive symptoms in the three-factor analysis. Rojo-Moreno et al. (1999) used a Spanish version of the EMBU (see paragraph on schizophrenia above). They found that a deficit in emotional warmth and a high level of rejection distinguished depressive patients from the controls. Runkewitz et al. (2006, see also above), using a German version of the EMBU, also found a lack of warmth and excess reaction in general-practice patients scoring high on depression on the HADS.

The **conclusion** is again that insecure attachment is a risk factor for depression, especially anxious attachment. Data on the influence of avoidant attachment are conflicting. Research with the PBI shows that depressive groups differ from the controls in that they generally perceive their parents as less caring and, although to a somewhat lesser extent, as over-protective.

Authors:	D/RD/NC/P	Mat. Low Care	Mat. Over-Prot.	Pat. Low Care	Pat. Over-prot.
Gotlib et al. 1988	DF	+	+	+	+
Ibid	RDF	-	+	-	+
Gotlib et al. 1991	PF	+	-	+	-
Boyce et al. 1991	PF	+	+	-	+
Vogel et al. 1997	D	+	+	+	-
Duggan et al. 1998	RD	+	-	+	-
Enns et al. 2000	DM	-	-	-	+
Ibid	DF	+	-	-	-
Lizardi & Klein 2002	D	+	+	+	_
Myhr et al. 2004	D	-	-	-	-
Total fem. clinical		7/9	5/9	5/9	3/9
Total males clinical		3/5	2/5	3/5	1/5
MacKinnon et al. 1993	NC	+	-	+	-
Neale et al. 1994	NCF	+	+	+	+
Patton et al. 2001	NC	+	-	+	-
Narita et al. 2000	NCM	+	-	+	-
Ibid	NCF	+	-	+	+
Martin et al. 2004	NC	+	+	+	-
Heider et al. 2006	NC	+	+	+	-
Total fem. non-clinical:		6/6	3/6	6/6	2/6
Total males non-clinical:		5/5	2/5	5/5	0/5
Grand Total :	F	13/15	8/15	11/15	5/15
	M	8/10	4/10	8/10	1/10

Table 1. Studies on the relationship between Parental Bonding Instrument and depression. In the second column D=depressed, RD=depression in remission, P=prospective study, NC=non-clinical group, F=female, M=male.

1.3.B.2.d Generalized anxiety disorder, panic disorder and phobias

Warren et al. (1997) reported from the Minnesota Longitudinal Study (Sroufe et al. 2005), a prospective study where 172 children were followed from birth until they were 17.5 years of age. Children that were ambivalently attached in the Strange Situation at one year of age were more often suffering from anxiety disorders at 17.5. Bosquet & Egeland (2006), however, reported on 155 subjects from the same Minnesota Longitudinal study and did not find an association between

insecure attachment and anxiety symptoms. They performed a path analysis, in which insecure attachment predicted negative peer relationship representations in pre-adolescence, which in turn predicted anxiety symptoms at 17.5 years of age. Fonagy et al. (1996) compared 82 non-psychotic inpatients with 85 matched controls with the AAI. Patients with anxiety disorders were more likely to be unresolved with regard to trauma or loss.
Bifulco et al. (2006) used the Attachment Style Interview in their investigation of 154 high-risk women, as mentioned above in the paragraph on depression. Moderate to marked insecure attachment predicted anxiety disorders. The fearful style was associated with social phobia, the angry-dismissive style with generalized anxiety disorders. Panic disorder and agoraphobia were not related to attachment style.

Hammen et al. (1995), using the Revised Adult Attachment Scale, found that the interaction of the Anxiety sub-scale score and inter-personal life events predicted anxiety symptoms. Eng et al. (2001) also used the RAAS and reported that secure and anxious attachment were over-represented in a sample of patients with social anxiety disorder, while the anxious attached patients showed more serious symptoms than the securely attached. Weems et al. (2002) used the Experiences in Close Relationships instrument (Brennan et al. 1998) to measure attachment in 527 high school and university students. Students with preoccupied and fearful attachment had higher scores on the Anxiety Sensitivity Index. The prospective studies by Hankin et al. (2005) mentioned in the paragraph on depression also revealed that anxious, but not avoidant attachment, predicted anxiety symptoms, again mediated by inter-personal and cognitive factors. In the same paragraph, the study by Runkewitz et al. (2006) is mentioned, in which general practitioner patients who had clinically relevant scores on the HADS were significantly more often insecurely attached, with the ambivalent style being more predictive then the avoidant style.
Pacchierotti and colleagues (2002) compared 22 panic disorder outpatients and 22 matched controls with the PBI. Panic patients perceived their parents as less caring than the controls did, while objective recall of parenting experiences did not differ.
In **conclusion**, there is evidence for the expected relationship between attachment anxiety, as measured in preoccupied/ambivalent classification, and high scores on the anxious attachment scale of two-

dimensional measures, and anxiety disorders. A study by Eng et al. (2001) even suggests that avoidant attachment protects against anxiety disorders.

1.3.B.2.e Post-traumatic Stress Disorder and Dissociative symptoms

Stovall-McClough & Cloitre (2003) administered the AAI to 52 adult victims of childhood sexual abuse. Fifty-five percent of the sample was classified as preoccupied and 75% as unresolved with regard to trauma. Subsequently (Stovall-McClough & Cloitre 2006) the same authors compared 30 female victims of childhood abuse with a diagnosis of PTSD with 30 childhood abuse victims without PTSD. The unresolved category was 7.5 times more likely to have PTSD. Riggs et al. (2007) applied the AAI in in-patients in a trauma treatment facility and correlated the results to PTSS and Dissociative Disorders. Unresolved trauma, according to the AAI, was associated with dissociation and PTSS. Muller and colleagues (2000b) reported that insecure attachment in the RSQ (Griffin & Bartholomew 1994) was highly over-represented in a volunteer sample of adults who reported childhood abuse. Especially fearful and preoccupied attachment were correlated to higher levels of symptomatology, even more than the history of physical abuse. Twaite and Rodriguez-Srednicki (2004) found that the relationship between childhood traumatization and the severity of post-traumatic stress symptoms due to the attack on the World Trade Center was mediated by Attachment Style Questionnaire confidence scale scores and Dissociative Experiences Scale scores. Fraley et al. (2006) found that securely attached high exposure survivors of 9/11 had fewer PTSD symptoms than those insecurely attached. Dismissively attached survivors were seen by their environment as not suffering from adjustment problems after the attack, while the subjects themselves reported high levels of PTSD. Finzi-Dottan et al. (2006) found that adolescents with learning disabilities had higher levels of PTSD following terrorist attacks than adolescents without learning disabilities. This higher level was partially mediated by anxious attachment, as measured by the Attachment Styles Classification Questionnaire.

Conclusions with regard to the relationship between traumatization, PTSD and attachment security have become easier to draw because of the studies that have been published in 2006. The fact that childhood

maltreatment predisposes to forms of insecure attachment seems logical, since attachment styles are adaptive in nature. That such an adaptation is not equally effective later in life, is demonstrated by the Twaite & Rodriguez-Srednicki study. Stovall-McClough & Cloitre's 2006 study suggests that the relationship between child abuse and later PTSD symptoms is partially mediated by attachment style. Because of the adaptive function of attachment strategies, it is also possible that the correlation between unresolved attachment and PTSD can be explained by a third common factor: the type or severity of abuse. Nevertheless, studies into the sequelae of terrorist acts suggest that anxious attachment increases vulnerability for PTSD.

1.3.B.2.f Obsessive-Compulsive disorder

Cavedo & Parker (1994) reported that higher scores on two measures for obsessiveness were correlated to paternal over-protection on the PBI in a non-clinical sample of 344 subjects. Vogel et al. (1997) did not find a difference between 26 OCD outpatients and 41 normal controls on the PBI and neither did Myhr et al. (2004) when comparing 36 OCD outpatients with 26 normal controls. Turgeon et al. (2002) compared a group of 43 OCD patients who had the 38 panic disorder with agoraphobia patients and 120 controls. The anxious groups did not differ amongst each other, but in comparison with the controls, they scored their parents higher on over-protection.
There are thus two negative studies with the PBI and two that find elevated scores for parental over-protection. These results differ from depression and eating disorders, where low parental care is the most prominent finding.

1.3.B.2.g Eating disorders

Fonagy et al. (1996) compared 82 non-psychotic in-patients with 85 matched controls with the AAI. Of the 14 eating disorder patients, one was classified as secure, nine as preoccupied and four as dismissive. Thirteen out of fourteen were unresolved with respect to loss or abuse. College women with dismissive attachment styles in the AAI had higher scores on an eating disorders questionnaires (Cole-Detke & Kobak 1996). In anorexia, both mothers of patients and the patients themselves showed an unusually high percentage of insecure attachment (93 and 85% respectively) as measured with the AAI (Ward et al., 2001). The dismissive pattern dominated, with 70% of

the insecure mothers and 79% of the insecure daughters being dismissive. There was no significant correlation between the styles of the mothers and the daughters. In a study of an ambivalently attached sub-group of a larger study, a surprise finding was that 7 out of 11 ambivalently attached women (according to the AAI) had eating disorders (Salzman, 1997).

Armstrong and Roth (1989) used the Hansburg Adolescent Separation Anxiety test (which contains pictures of separation situations, Hansburg 1986) to determine attachment style in a group of 27 hospitalized women with eating disorders. They found that only one of them was not anxiously attached. Becker et al. (1987) investigated college students with a self-report inventory of bulimia and the insecure attachment sub-scale of the Bell Object Relations Inventory (Bell et al. 1986). They found a positive correlation between the severity of eating disorders and insecure attachment. Heesacker & Neimeyer (1990) also carried out a study among college students with the Bell Object relations Inventory and found a positive correlation between insecure attachment and the Eating Attitude Test (Garner & Garfinkel 1979) and the Eating Disorder Inventory (Garner 1983). Kenny & Hart (1992) found that 68 eating disorder female in-patients differed from college women, in that the patients scored lower on scales of the Parental Attachment Questionnaire (Kenny 1990) concerning affective quality of attachment, parental fostering of autonomy and parental role in providing emotional support. Brennan & Shaver (1995) used both categorical and dimensional self-report attachment measures in a group of college students and found that preoccupied attachment was correlated to scores on the Eating Disorder Inventory. Friedberg & Lyddon (1996) found preoccupied attachment, as scored with the Relationship Questionnaire (Bartholomew & Horowitz 1991), was more present in a group of women with eating disorders than in college controls. Chassler (1997) used the Attachment History Questionnaire (Pottharst 1990) to compare in-patients with social work students and found that patients experienced their parents as less responsive, available or trustworthy. When confronted with the Adult Attachment Style questionnaire (Bartholomew & Horowitz 1991), women with greater eating problems had lower scores on closeness and dependency sub-scales and higher scores on anxiousness scales (Evans & Wertheim 1998). Ward et al. (2000) report that eating disorder patients scored higher on the sub-scales of Compulsive Care Seeking and Compulsive Self Reliance than the controls when administered the Reciprocal

Attachment Questionnaire (West & Sheldon-Keller 1992). A study of 145 female outpatients aged 18-24 years and 315 women from the population register Broberg et al. (2001) found a correlation between insecure attachment (Relationship Scales Questionnaire) and eating disorders, both in the clinical population and in the controls, the latter group referring to eating disorders in the past. Pierrehumbert et al. (2002)**,** using a self-report Q-sort questionnaire (CaMir, Pierrehumbert et al. 1996) designed to classify according to Main's sub-types of attachment, found that eating disorder patients had a preoccupied attachment style more often than the controls. Zechowski et al. (2004), using a Polish version of the Inventory of Parent and Peer Attachment, found that attachment was disturbed in eating disorder adolescents, as compared with healthy controls. Troisi et al. (2005) used the ASQ to compare 78 women with eating disorders with 64 healthy controls. Anxious attachment was significantly associated with eating disorders, avoidant attachment was not. There was no difference between the different types of eating disorders. Hodson et al. (2006) did not find a correlation between a Healthy Current Attachment measure (derived from the Collins & Read Attachment Scale) and bulimic behaviors in a community sample of adolescent Latinas. Among American female college students, attachment avoidance and, to a lesser extent, attachment anxiety (as measured by a modified version of the Experiences in Close Relationships questionnaire) predicted psychological correlates of eating disorders (Kiang & Harter 2006). A relationship between preoccupied scores on the Adult Attachment Scale and disordered eating was found to be mediated by neuroticism in a female sample from the Michigan State University Twin Study (Eggert et al. 2007). The Relationship Questionnaire was used by Lehoux and Howe (2007) in a comparison between bulimic patients and their non eating disordered sisters. Insecure paternal attachment yielded an Odds Ratio of 9.9 for the risk of developing bulimia.

The Parental Bonding Instrument has often been used in research on eating disorders (see table 2). In clinical groups, the majority of the studies in anorexic and bulimic groups show that maternal care is perceived to be lower than in the controls, with a substantial minority of studies showing evidence of perceived maternal over-control. The fathers are not perceived differently compared with controls in anorexic patients. Bulimic patients, however, have perceived their fathers low in care in most of the studies and also seem to perceive more paternal over-protection than the anorexic patients. When

comparing restricting anorexia nervosa patients with the binge-purging type, Di Pentima et al. (1998) found the same difference. The majority of non-clinical groups that score high on eating disorder measures do not rate their parents differently from the controls. Castro et al. (2000) adapted the EMBU for adolescents, so that it also contains information about the present. The only difference they found between an adolescent group of female anorexia patients and a control group was that the patients perceived more emotional warmth from their fathers. On the other hand, when the patients were divided into groups with good and bad outcomes, perceived rejection by the father was by far the strongest predictor of a bad outcome.

In **conclusion**, the evidence for an association between insecure attachment and eating disorders is overwhelming, both in self-report and in interview measures. In self-report the association between the preoccupied sub-type and the severity of disorder is the strongest, while in the AAI both dismissing and preoccupied attachment are more frequent than in the controls. Strangely enough the difference between anorexic and bulimic type of patients that surfaces so clearly in the PBI is not present in the categorical or dimensional measurement of attachment in the stricter sense. This is probably due to the fact that non-PBI studies generally did not distinguish between clinical sub-types and between maternal and paternal attachment.

Authors	C/NC	Type of problem	Mat. Low Care	Mat. Over-prot.	Pat. Low Care	Pat. Over-prot.
Gomez 1984	C	AN	+	+	-	-
		B	-	-	+	-
Palmer et al. 1988	C	AN	+	-	-	-
		B	+	-	+	-
Pole et al. 1988	C	B	+	-	+	-
Calam et al. 1989	C	B	+	+	+	+
Steiger et al. 1989	C	E	-	-	+	-
		B	-	-	+	+
Russell et al. 1992	C	AN	-	-	-	-
Rhodes & Kroger 1992	C	E	-	+	-	-
Sordelli et al. 1996	C	AN	-	-	-	-
		B	-	+	-	-
Laporte et al. 2001	C	AN	+	+	-	-
Wade et al. 2001	C	B	+	+	-	-
De Panfilis et al. 2003	C	E	+	+	+	+
Canetti et al. 2008	C	AN	+	-	+	+
Total Clinical		AN	4/6	2/6	1/6	1/6
		B	4/7	3/7	5/7	2/7
		E	1/3	2/3	2/3	1/3
Lavik et al. 1991	NC	E	-	+	-	-
Kent & Clopton 1992	NC	E	-	-	-	-
Haudek et al. 1999	NC	E	+	-	-	-
Meyer & Gillings 2004	NC	B	-	-	-	+
Total NC			1/4	1/4	0/4	1/4
Grand Total:		AN	4/6	2/6	1/6	1/6
		B	4/8	3/8	5/8	3/8
		E	2/6	3/6	2/6	1/6

Table 2. Overview of studies on eating disorders with Parental Bonding Instrument (PBI). C/NC refers to clinical and non-clinical groups. AN/B/E refers to diagnostic classification or type of instrument used, AN=Anorexia Nervosa, B=Bulimia, E=Eating disorders.

1.3.B.2.h Substance abuse

Schindler et al. (2005) review the literature on attachment and substance abuse and report that the AAI was used in three studies in (adolescent) psychiatric inpatients and one study in non-clinical subjects. Substance abuse was correlated to dismissing attachment in two studies, with preoccupied attachment also in two and with

unresolved attachment in two as well. Caspers et al. (2006) later found that all three insecure types of attachment they measured with the AAI (dismissing, preoccupied or earned-secure) were more prone to alcohol or drug abuse than the continuous-secure group. Unresolved attachment did not predict abuse. Riggs et al. (2002) used the AAI in expectant parents and found that none of the four attachment categories was related to substance abuse.
In self-report scales using the three-type Hazan & Shaver instrument, avoidant attachment is related to alcohol abuse and the severity of alcohol abuse. Only Vaz-Serra et al. (1998), using the Revised Adult Attachment Scale, found that anxious attachment was correlated to alcoholism. With the four-factor Bartholomew instrument, however, one study showed a relationship between fearful and preoccupied attachment and alcohol abuse and one study only between preoccupied attachment and alcohol abuse. A later study with the Bartholomew instrument (Vungkhanching et al. 2004) showed a relationship between fearful and dismissive attachment and alcoholism, while a positive family history was also correlated to these two avoidant styles. Schindler et al.'s own study (2005) showed a significant over-representation of fearful attachment in drug-dependent adolescents as compared with non-clinical siblings. Elgar et al. (2003) asked 68 male juvenile delinquents to complete self-report measures on attachment, substance use and behavior problems. Insecure attachment was correlated to both substance use and behavior problems. McNally et al. (2003) found a correlation between a negative model of the self in a one-item inventory of the Bartholomew and Horowitz four-factor model and alcohol abuse in college students. Hodson et al. (2006) report on a negative correlation between a Healthy Current Attachment measure (derived from the Collins & Read Attachment Scale) and polysubstance abuse in adolescent Latinas.
Gomez (1984) found that alcoholics experienced their fathers as less caring and alcoholic males scored their fathers lower than the controls on the PBI over-protection factor. Alcoholic females experienced their mothers as more caring. Gerra et al. (2007) also used the PBI when comparing cocaine-addicted individuals with controls. The cocaine users scored both their parents lower on care.

In **conclusion**, research into attachment and substance abuse has showed an association between insecure attachment and substance abuse. There is no consistent pattern with regard to the type of

insecure attachment. This may be due to the use of different instruments in different age groups abusing different substances.

1.3.B.2.i Somatisation/hypochondriasis/medical consumption

Taylor et al. (2000), using the ASQ (Hazan & Shaver 1987), found that those attending primary care, who presented with unexplained physical symptoms, were more often insecurely attached than those presenting with organic symptoms. Those presenting with overt psychological symptoms were more often insecurely attached than those presenting with physical symptoms, unexplained or organic. Ciechanowski et al. (2002b) investigated the association between symptom reporting and attachment classification (as measured by a combination of the RQ – Bartholomew & Horowitz 1991 – and the RSQ – Griffin & Bartholomew 1994) in a group of female HMO members. They found that both preoccupied and fearfully attached women reported more physical symptoms. Care cost and utilization, however, differed between these two attachment styles. Preoccupied women had higher costs than the other two attachment groups, while fearfully attached women had lower costs. This was after correction for physical illness severity and depression. Zech et al. (2006), in patients visiting their general practitioner, found that anxious attachment, as measured with a renewed factor orientation of the Adult Attachment Scale questions, was positively correlated to depressive affect, while avoidant attachment was negatively correlated to it. Avoidantly attached patients were more likely to report physical symptoms than mental symptoms, and health symptoms that were more severe than the mean, while anxiously attached patients reported less severe problems. The same group (Ciechanowski et al. 2002c) found an association between life-time unexplained physical complaints in Hepatitis-C patients and preoccupied and fearful attachment. Wearden et al. (2005) confirmed the results of Ciechanowski and colleagues, in that male and female undergraduate students with a preoccupied or fearful attachment style reported more anxiety and depression-related somatic symptoms than the other two classifications. Hoermann et al. (2004) investigated several possible predictors of medical hospitalizations in females with cluster B personality disorders. Attachment style, as measured by the Relationship Questionnaire (Bartholomew & Horowitz 1991)and especially preoccupied attachment, predicted 23% of the variance in the length of hospitalization, more than the severity of

psychopathology or level of psycho-social functioning did. Noyes et al. (2003) found in a general medical population that insecure attachment measured with the Relationship Questionnaire, especially preoccupied attachment, predicted high scores on a hypochondriasis measure. Waldinger et al. (2006) used the Relationship Scales Questionnaire to see if attachment mediated the relationship between childhood trauma and adult somatization. In women, fearful attachment fully explained this link, while in men, insecure attachment did not mediate this, but contributed on its own to adult somatization.

The **conclusion** is that insecure attachment, especially preoccupied or anxious attachment, is a risk factor for unexplained physical symptoms. This may be the result of increased physical symptoms as a manifestation of increased anxiety (see also the paragraph on anxiety disorders) or of increased need for reassurance as a result of anxiety or of increased proximity seeking to a substitute attachment figure, i.e. the doctor.

1.3.B.2.j Medical conditions

Ciechanowski et al. (2002a) reported that dismissive attachment, as measured with a combination of the RQ and the RSQ, was associated with poor diabetes control as apparent in HbA1c levels.
Picardi et al. (2005) compared a group of psoriasis patients with recent exacerbation with a group of dermatology patients with disorders that were not considered to be strongly influenced by psycho-social components. On the Experiences in Close Relationships anxiety scale, patients scored higher than controls, while there were no differences in the avoidance scale. In ulcerative colitis, depression often accompanies active disease. Maunder et al. (2005) found that in the low, middle and higher tercile of attachment anxiety scores, there was an increase in significance of correlation between depression and the activity of disease, meaning that attachment anxiety serves as a mediator. In attachment avoidance score terciles, only the highest yielded a significant correlation between disease activity and depression. Merskey et al. (1985) did not find differences in PBI scores between 103 neurological outpatients with headaches not due to significant illness and a general practice population. Rossi et al. (2005) used the Attachment Style Questionnaire to investigate a group of migraine patients. They found that lower attachment confidence

was correlated to more severe disability in the group with episodic migraine, but not in the group with chronic migraine.
The **conclusion** that can be drawn on the basis of these few reports is that anxious attachment is correlated to the activity of physical illnesses, with prospective studies needed to determine direction of causality. Dismissive attachment possibly leads to denial of suffering and hence to poor diabetes control.

1.3.B.2.k Personality Disorders
Agrawal et al. (2004) reviewed 13 studies that examined attachment types in patients with borderline personality disorder (BPD) or characteristics of it. Their conclusion was that attachments in BPD "seem best characterized as unresolved with preoccupied features in relation to their parents, and fearful or, secondarily, preoccupied in their romantic relationships". They argue, however, that the diagnostic feature of BPD of intense and unstable relationships is so similar to the description of preoccupied attachment that the relationship may be circular. Levy (2005) also reviewed the literature on BPD and attachment and concluded that, although insecure attachment is the rule, interview measures do not yield a specific insecure type. Self-report measures, however, consistently show a positive correlation to fearful avoidant and preoccupied attachments. Rosenstein & Horowitz (1996), as mentioned in the paragraph on general psychopathology, correlated AAI classification to psychopathology in adolescent in-patients. Dismissing attachment was correlated to conduct disorder, narcissistic and anti-social personality disorder and narcissistic, anti-social and paranoid personality traits. Preoccupied attachment correlated to obsessive-compulsive, histrionic, borderline or schizotypal personality disorder, and avoidant, anxious, and dysthymic personality traits. Riggs et al. (2007), in the study mentioned in the section on PTSS and dissociation, found that both unresolved loss and unresolved trauma in the AAI contributed to schizotypical and borderline personality scores.
Brennan & Shaver (1998) administered measures of attachment and of personality disorders to a group of 1407 college students. Attachment insecurity was over-represented in those with personality disorders. They also found evidence for a two-dimensional space, in which personality disorders and attachment patterns could be arranged. Only a psychopathy-like factor in personality disorders could not be accommodated. A limitation of the study was that the personality

disorder instrument (Personality Disorders Questionnaire-Revised, PDQ-R) yielded a percentage of 75% with a personality disorder in this non-clinical sample. Bender et al. (2001) used the MMPI to measure personality pathology and the Reciprocal Attachment Questionnaire for attachment. They found that insecure attachment was strongly related to cluster-B personality pathology, with separation protest, fear of loss, proximity seeking and perceived unavailability – in that order – as specific dimensions. This pattern differed considerably from the pattern in cluster A (with only 'lack of use' significantly related) and cluster C (only 'fear of loss' significantly related) personality pathology. Within the B cluster, the borderline personality had the greatest association with attachment dimensions, followed by anti-social, narcissistic, histrionic and aggressive/sadistic traits. Westen et al. (2006) devised the Attachment Prototype Questionnaire, to be used by clinically experienced observers. In adolescents, dismissing attachment was correlated with schizoid personality, disorganized with schizotypal and preoccupied and disorganized attachment both strongly with borderline personality. In adults, dismissing attachment was strongly correlated to schizoid and narcissistic personalities, preoccupied with borderline and histrionic and disorganized and to paranoid and borderline personalities. Crawford et al. (2006) developed attachment scales for the prospective Children in the Community Study where subjects were followed from age 16 until 33. The CIC attachment instrument corresponds to the Experiences in Close Relationships Scale. Anxious attachment was correlated to cluster B and C symptoms and avoidant attachment to cluster A. Timmerman & Emmelkamp (2006) studied the relationship between personality disorders and Relationship Questionnaire scores in prisoners, forensic in-patients and controls. They report an association between preoccupied attachment and borderline personality and between anti-social personality and dismissing style. Minzenberg et al. (2006) compared borderline patients with controls on the Experiences in Close Relationships questionnaire. The borderline group had higher scores on attachment anxiety.

A number of studies have been conducted on borderline patients with the Parental Bonding Instrument. Borderline patients scored their parents lower on care (Paris & Frank 1989; Zweig-Frank & Paris 1991; Torgersen & Alnaes 1992) and higher on over-protection (Zweig-Frank & Paris 1991; Torgersen & Alnaes 1992). Byrne and

colleagues (1990) compared PBI scores of 15 borderline patients with normal population data. Borderline patients perceived both their parents as more over-protective and less caring. Nickell et al. (2002) found that borderline factor scores were negatively associated with maternal care and positively associated with maternal over-protection and autonomy encouragement. Paternal PBI scoring was not associated with borderline factors. These borderline factor scores were also negatively associated with secure adult attachment and positively with ambivalent attachment, measured according to Hazan & Shaver (1987). Torgersen & Alnaes (1992) assessed schizotypical people and found that they scored their parents lower on care than the controls, but that they also remembered under-protection, instead of the over-protection found in the same study in borderlines. A study of 742 community-based subjects in the U.S. (Reti et al., 2002) showed that anti-social traits in men were correlated to low maternal care and high maternal over-protection, while anti-social traits in women were associated with low paternal care and high maternal denial of psychological autonomy. This was measured according to the three-factor structure in the PBI. Nakash-Eisikovits (2002) asked 294 psychiatrists and psychologists to complete a number of measures on a patient that were in their treatment for maladaptive personality patterns. On the clinician-report attachment questionnaire disorganized attachment correlated to various forms of personality pathology, preoccupied attachment with withdrawal, internalization and introversion and dismissive attachment was not correlated to any single form of personality pathology.

In **conclusion**, studies in personality disorders consistently reveal attachment disturbances, with borderline patients being characterized as unresolved and preoccupied. This is in agreement with the high percentage of childhood trauma (e.g. 81% in Herman et al. 1989) that is mentioned in other studies. Furthermore, the results of PBI research with rather consistent reports of both low care and high over-protection are in agreement with the push and pull type of interpersonal behavior. Research in other personality disorders is too scarce for definitive statements to be made.

1.3.C Attachment and psychopathology: general conclusion.

Psychopathology in general is more prevalent in people with insecure attachment than in people with secure attachment, both in cross-sectional and in longitudinal studies. In children, disorganized attachment predisposes to both internalizing and externalizing symptomatology. In adults, there has as yet been very little research into the corresponding "unresolved" classification. The available data suggest a role in trauma-related disorders like post-traumatic stress disorder and borderline personality disorder, although this runs the risk of being tautological, given the traumatic origin of the unresolved category. Ambivalent/preoccupied attachment is associated with internalizing scores on the Child Behavior Check List in children and anxiety, depression, eating disorders, somatization and borderline personality in adults. Avoidant/dismissive attachment has an unclear relationship with psychopathology, with weak evidence for involvement in both internalizing and externalizing disorders. However, there is some evidence that avoidant/dismissive attachment leads to an under-reporting of disease symptoms and a reduction in help-seeking behavior in general, with the risk that the role in psychopathology is underestimated.

The bond with the parents is perceived as being less satisfactory in people with psychopathology than in the controls. In particular, the lack of parental care is a frequent finding, with parental over-protection present predominantly in schizophrenia, depression and borderline personality disorder.

Although there is abundant evidence of the involvement of attachment in psychopathology, relatively little use is made of these findings in clinical practice. Probably the main reason for this is that the diagnosis of attachment status has as yet no therapeutic implications, with the exception of very young children. Indeed, clinical use of attachment theory in child psychiatry is much more frequent than in adult psychiatry. With the absence of a biological underpinning and of therapeutic consequences, the acceptance of attachment theory is more limited than would be expected purely on the grounds of the scientific evidence for the involvement in psychopathology.

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CHAPTER 2

A review of preference studies in rodents.

The behavioral part of this review is limited to research that was directed at behavior that indicates a preference for parental figures or for stimuli that had become associated with them. Data regarding pup behavior directed at the acquisition of nourishment or warmth regardless of the provider were not included. Also, the wealth of reports on isolation or separation experiments during infancy was excluded, unless the research was aimed at differentiating the stress reducing properties of attachment-related stimuli during isolation. For practical reasons, only behavioral research in rodents is discussed. The pharmacological part of this chapter also reviews other species and adult animals.

2.1 Rats

2.1.A Perinatal period (PostNatalDay 0-4).

Rat pups delivered through Caesarean section preferred amniotic fluid of their own mother to that of an unrelated female immediately after birth (Hepper, 1987). Teicher and Blass (1977) washed the nipples of parturient dams, smeared different substances on them and then measured nipple attachment by the newborns. Neither various chemicals, nor the mother's urine, nor virgin female saliva could reinstate the nipple attachment that had ceased after washing. Only the mother's saliva or her amniotic fluid could. The same group (Pedersen and Blass 1982) exposed pups pre- and post-natally to citral, then washed the nipples of anesthetized parturient dams and scented them with citral and registered the occurrence of first-time suckling. Pups suckled the citral scented nipples but not the normal unscented nipples. Neither pre- nor post-natal exposure alone was sufficient to elicit suckling.

Another form of this maternally-directed orienting behavior (Polan & Hofer 1999) is the goal-corrected behavior aimed at ventral-ventral contact (Polan 2005). This behavior can be seen as early as an hour after birth and differs in intensity when confronted with a piece of cotton or with the mother's skin. This difference increases during the first two days (Polan et al., 2002).

Rat pups are able to discern home shavings from fresh shavings on Postnatal Day (PND) 1 (Singh et al., 1977), even as early as 8 hours after birth (Hepper 1987). Sczerzenie and Hsiao (1977) found a preference for home shavings as opposed to fresh shavings on PND 2.

Schapiro and Salas (1970) studied spontaneous activity in 2 to10-day-old rats and saw that it was reduced in the presence of maternal odor.

2.1.B Infancy (PND 4-10)

Duveau and Godinot (1988) added four different artificial odors to the dam's food and tested the pups for odor preference. From day 1 until day 6, pups preferred the odors they were raised in. Terry & Johanson (1994) induced a preference for eucalyptus in pups that were raised by a mother fed on a diet containing eucalyptus. The preference was stronger than when the pups were raised in a room where the smell of eucalyptus circulated but their mother was fed a normal diet. The preference was almost gone on PND 12. Through cross-fostering procedures they established that the preference was largely due to post-natal experiences.

Cornwell-Jones and Sobrian (1977) described an attraction in 4-day-old pups for shavings from the home cage, an attraction that grows stronger during the first post-natal week. However, rat pups on PND 4 were also attracted to shavings from a strange cage when compared with fresh shavings. Polan & Hofer (1998) offered rat pups a choice between a chamber with their mother (awake or anesthetized) and a chamber with home shavings. Pups preferred the maternal odor on PNDs 5, 7 and 9, particularly if they had been deprived of their mother overnight before testing. A temperature gradient (with the mother's side being slightly warmer) increased the preference and even induced a preference as early as PND 2.

Ultrasonic vocalizations (USV) are sounds emitted by rodent pups at a frequency of 40kHZ or more when they are isolated or cold. They induce retrieval by the mother (see Hahn & Lavooy, 2005, for a review) and are thus also considered a form of the maternally-directed orienting behavior mentioned above (Polan & Hofer 1999). Oswalt & Meier (1975) placed pups on PND 3-13 in empty dishes or dishes filled with clean bedding or soiled bedding from the home nest. Pups from all ages emitted fewer USVs when placed in dishes with bedding as compared with empty dishes. From PND 5 upwards, pups reduced their USVs in home nest bedding as compared with clean bedding. This was replicated by Conely & Bell (1978). Kehoe & Blass (1986) isolated 10-day-old pups for 5 minutes from their mother and then placed an anesthetized female with them. This induced a significant reduction in USVs. Home shavings also induced the decrease, whereas fresh shavings did not. Shair et al., (1997) registered

ultrasonic distress vocalizations in 8, 11 and 12-day-old pups in reaction to the absence and presence of an anesthetized adult rat. They isolated pups in cold temperature, then placed their anesthetized dam or an anesthetized male with them, subsequently removed the anesthetized animal and measured USVs. There was no difference in the reaction to the placement in the cage; both dam and male induced a great reduction in the USVs that were emitted because of isolation and temperature. Removal of the dam, however, induced an acute increase in USVs, while USVs remained low in reaction to the removal of the male. This is called USV potentiation (Hofer et al., 1994): when pups are isolated they emit USVs and stop doing this when their dam is placed with them. When the dam is removed after 1 minute of reunion, the rate of USVs becomes twice or three times higher than during the previous isolation period. This potentiation is influenced by the duration of isolation and reunion and by the temperature in the room (Shair et al. 2003). The potentiation phenomenon can be induced by their own mother, by virgin females or by adult males, provided these latter two were present with dam and pup when the pup was being reared (Brunelli et al., 1998). Although the reduction in isolation-induced USVs can be caused by a great number of living stimuli, like a single other pup (Hofer & Shair 1978) or anesthetized strange males, even if the pups are raised in isolation from birth (Hofer et al., 1989), the potentiation phenomenon is limited to attachment-related cues.

Teicher and Shaywitz (1979) studied activity directed towards different conspecifics and objects in 8 to9-day-old rats. Their natural mother, another lactating female, a virgin female, a male rat and wooden blocks or plastic container elicited activity of successively diminishing intensity. Compton et al. (1977) found that the deceleration in the heart rate following a 2-hour separation in 8-day-old rat pups was interrupted by the presence of maternal odors, in contrast to the odors of non-lactating females or neutral controls.

Haroutunian & Campbell (1979) found that before PND 10, pairing of an external aversive stimulus (i.e. electrical shock) with an odor did not result in an odor aversion. They also showed that there was a difference between external (the peripheral shock) or internal (intraperitoneal shock or lithium poisoning) aversive stimuli. Internal stimuli resulted in odor aversion before PND 10 as well. If the internal stimulus (i.e. a toxic substance) was coupled to milk received during

nursing, a taste aversion did not develop in pre-weaning rats even after PND 10 (Martin & Alberts, 1979). Taste aversion did develop, however, if the same milk was given via a cannula by the experimenter. Sullivan et al. (1986) discovered that a mildly aversive odor (orange extract) can be preferred by 3 and 6-day-old rats if coupled to maternal saliva or even to an aversive stimulus like tail-pinching.
Roth & Sullivan (2005) showed that semi-natural maternal maltreatment (induction of stress in the dam that leads to stepping on the pups, dropping them, pushing them away etc.) also resulted in an odor preference on PND7-8 when offered paired with a peppermint odor. Results did not differ between normal interaction with the dam and maltreatment.

2.1.C Pre-weaning period (PND 10-21)
Fillion & Blass (1986) induced a preference for lemon scented dams on PND 10 when pups were raised with dams that had been treated with the lemon scent daily. Pre-natal exposure to garlic (which was fed to the mother during pregnancy) resulted in a preference for garlic at PND 12, although there had been no exposure to garlic since birth (Hepper 1988).
Nyakas & Encroczi (1970) let 10-day-old rats choose between a goal box in a U maze containing their own mother and a goal box containing a non-lactating female. The mother was preferred above the non-lactating female, which was preferred above a male.
Shayit & Weller (2001) gave 11 to12-day-old rats a choice between an empty arm and an arm that contained their mother's faeces. Rats preferred the maternal odor arm. Willford et al. (1999) tested rats reared in a different manner on PND 15. Pups were reared artificially from PND 4 to PND 10 or otherwise and received different dosages of cocaine or saline during rearing. All groups preferred the home cage odors to clean shavings.
Experiments by Leon and Moltz (1971, 1972) showed that 14 to 27-day-old Wistar rat pups, placed in an air stream blown through two compartments, preferred their mother's compartment to the compartment of a nulli-parous female. This was replicated by Holinka & Carlson (1976) for Sprague-Dawley rats. In the experiments by Leon and Moltz, the pups did not discriminate between their mother's odor and the odor of a strange lactating female. In addition, they preferred the strange lactating female to the nulli-parous female. Because they also prefer their mother's odor in an air stream test

above the odor from 5 litter mates at PND 16 (Leon 1974), the presence of a maternal pheromone was suggested that would equally attract pups from different nests. Gregory and Pfaff (1971) reported that rat pups of PND 12-19 preferred home litter shavings over fresh shavings and over shavings from a non-pregnant female rat. They did not prefer home litter shavings to other litter's shavings, in line with the maternal pheromone hypothesis. Carr et al. (1979), however, found that 12-day-old rat pups preferred bedding from the home nest to bedding from a nest of the same age, while 16-day-olds had no preference and 20-day-olds preferred the strange bedding. Hepper (1986) found a continuous preference in an air stream test for a chamber containing their own mother as opposed to a chamber containing a strange mother from PND 5 until the end of testing on PND 22. Differences between the results of Hepper and Leon & Moltz could be explained by the procedure. Leon & Moltz isolated their pups 3 hours before testing, which may lead to a reduction in body temperature and less discriminate behavior. Hepper isolated his pups for only 30 minutes. Another explanation could be the construction of the test apparatus, with Leon & Moltz using Y-shaped and Hepper T-shaped apparatus.

Whatever the explanation, the fact that two authors report discrimination between the mother and another lactating female leads to the conclusion that there is no evidence for the existence of a non-specific maternal pheromone with general attractive properties, but rather that there is a specific signal from the dam that is recognized by the own pups. Hepper (1986) also showed a preference for the father on PND 10-14, which switched to a preference for a strange male at PND 18-22. This pattern was even present if the father had been removed from the nest before birth.

Randall and Campbell (1976) found that isolation-induced hyperactivity in 15-day-old rats was reduced by the presence of siblings and of anesthetized lactating females and anesthetized male rats. In a very fine study, Moriceau & Sullivan (2006) investigated the influence of the presence of the mother on odor-shock conditioning. PND 8 rat pups acquired an odor preference through odor-shock conditioning, with or without the presence of the anesthetized mother, PND 12-15 pups only when odor-shock conditioning occurred in the presence of the mother and PND 21-23 pups learned odor avoidance. The presence of the mother caused a reduction in the corticosteroid levels in the pup, which suppressed amygdala activity. Exogenous corticosteroids could activate the amygdala, leading to the learning of

an odor aversion in 12 to15-day-olds, even when the mother was present.
Rat pups avidly seek tactile contact with their mother and they also do so with litter mates (huddling). Alberts and his colleagues extensively researched huddling behavior (Alberts 1978a; Alberts & Brunjes 1978b; Brunjes & Alberts 1979; Alberts & May 1984; Alberts 2007). In the first two weeks, rat pups huddle with anything that is warm. Later, olfactorial characteristics become more important than thermal. Pups that were raised from PND 1 to PND 20 with a chemically-scented dam, or with a dam that was not scented, developed a preference by PND 15 to huddle with conspecifics that had the olfactory characteristics of the rearing environment.

2.1.D Weaning and post-weaning period (PND 21-60)
Marr and Gardner (1965) rubbed cologne, methyl salicylate or normal (nest) odor on the fur coats of rat pups and their mothers from PND 2 until PND 30. On PND 30-45, pups were tested in apparatus that contained stimulus animals from the three different odor groups. The cologne and nest odor-treated pups preferred the stimulus animal with the odor they had been treated with, while the methyl salicylate group did not show a preference. When the animals had become sexually mature, they were given a breeding test. Cologne-treated animals showed reduced activity in contact with a normal stimulus animal. The same group (Marr and Gilliston, 1970) rubbed rat pups daily with a cloth containing ethylbenzoate, acetophenone or neutral odors. Exposure to the odors was done in three different time periods: PNDs 3-10, 11-18 and 22-29. Pups were tested in an odor preference test between PNDs 45-60. Preference for the experimental odor was greatest when exposure had taken place on PND 3-10 and absent when exposure was on PND 22-29. Galef & Kaner (1980) exposed rat pups to a mildly aversive odor (peppermint) and tested them for preference for it**.** Simple exposure from PND 0 to PND 33 did not result in a preference, but smearing it on the dam did. Sullivan et al. (1990) raised pups up to PND 19 with dams fed normal diets, odor-suppressing sucrose diets or sucrose diets with peppermint addition. Pups showed a preference and enhanced olfactory bulb neural response when confronted with the odors they were reared in. Brake (1981), however, already noticed that pups suckling a passive dam which was not their own mother, learned a preference for the odor of the shavings in which suckling took place . This means that odors that become associated with the suckling situation are preferred, regardless

of the origin. Leon et al. (1977) isolated pups 3 hours/day from PND 1 to PND 19 and exposed them to the odor of maternal excreta and to peppermint and lemon odors with odors from an empty box as controls. On PND 20, pups had a preference for a goal compartment or food with the odor to which they had been exposed. Leon et al. conclude that the presence of the mother or the litter mates is not necessary for the development of a preference for an odor of any kind, it is simply familiarity (or a reduction in the aversion to novel stimuli) that causes the preference. An experiment by Cromwell et al. (2007), however, contradicts this. They induced a preference for a lemon-scented compartment as opposed to a non-scented compartment on PND 14 through three thirty- minute conditioning trials on PND 13. Rat pups that had been conditioned to their lemon sprayed dam, developed the preference, while pups conditioned with a lemon-sprayed cotton ball did not.

2.2 Other Rodents

Porter and Ruttle (1975) found that 1-day-old **spiny mice** (Acomys cahirinus) preferred bedding from their home cage to clean bedding and to bedding from a nulli-parous female. They did not prefer their own bedding over bedding from another litter's cage. Porter and Doane (1976) offered 1-day-old spiny mice a choice between bedding soiled by a lactating female (not the biological mother) or the bedding soiled by an adult male or a litter of neonatal conspecifics. They preferred the bedding of the lactating female. The same group (Porter et al.,1978) offered 15 to 25-day-old spiny mice a choice between bedding soiled by a lactating female (not the biological mother) and bedding soiled by a non-lactating female. Mice15 and 20 days old preferred the odors of the lactating female and 25-day-old mice the non- lactating female. Later, Porter et al. (1990) tested the response of spiny mice weanlings to food items that had been partially eaten by different types of conspecifics. Prior contact with the food by the mother induced greater consumption of it than prior contact by the father or an unfamiliar mother. Porter and Etscorn (1974) devaluated the attachment importance of experiments with artificial odors by showing that spiny mice exposed to cinnamon or cumin 2 to12 hours after birth preferred this odor 24 hours later, even if the exposure had

taken place in the absence of association with any maternal or feeding reinforcers.

Breen & Leshner (1977) found that **mice** (Mus musculus) preferred their own mother and her droppings to a virgin female and her droppings. They did not find this preference in a comparison with another lactating female, although the data suggest this might be due to a type II error. The preference for the mother was in any case less strong than when the mother was compared with the virgin female. Marchlewska-Koj et al. (1999) elicited ultrasonic vocalizations in three-day-old CBA mice by cooling them down. The effect of different odors on number, frequency and duration of USVs was measured. Clean bedding, bedding from the home cage and from a CBA nest did not differ from each other. Bedding from a C57BL nest elicited more calls of longer duration than the other three, even if the CBA pups had been cross-fostered to a C57BL mother immediately after birth. The frequency of the reaction to CBA bedding was higher in the CBA raised group than in the group that had been cross-fostered to a C57BL mother.

Devor and Schneider (1974) tested preferences for cage beddings from different origins in **golden hamsters** (Mesocricetus auratus). From PND 7-8 onwards they preferred shavings from the home cage over fresh shavings and over shavings from male and non-lactating female hamsters. They did not prefer their own home shavings over those from another dam and her litter. Gregory and Bishop (1975) performed comparable experiments with comparable results, although they did not find a preference for home shavings over those from a non-lactating female.
Carter (Carter & Marr 1970, Carter 1972) described the way that **guinea pigs** (Cavia porcellus) preferred the unnatural odors of ethyl benzoate and acetophenone in which they were reared to normal odors. Porter et al. (1973a) measured the time guinea pigs spent in a compartment of an open field adjacent to a cage where the rest of their litter and their mother were kept in the experimental condition and which was empty in the control condition. The time spent in the experimental condition during the first month was significantly longer than in the control condition. The same authors (Porter et al. 1973b) did not find a preference in infant guinea pigs when they were offered a choice between their biological mother and another lactating mother with which they had also been raised. The infants, however, tended to

spend more time nursing and in proximity to their biological mother when in the same compartment with the other lactating mother (Fullerton et al., 1974). Pettijohn (1979a) placed the father and the mother of guinea pig infants in separate boxes, separated by wire mesh from a compartment where the infant was observed for 5 minutes. Infants spent more time in the square closest to the mother when 2 and 4 weeks old, but no more time when 6 and 8 weeks old. The time they spent near their father did not differ from chance.
The same author (Pettijohn 1979b) measured distress vocalizations when infant guinea pigs were separated from their mother and/or their home environments. Separation from the mother in the home environment caused a level of distress vocalizations between the high level when isolated from both home environment and mother and the low level when the mother was present, be it in the home environment or in the novel environment. Pettijohn also measured the vocalizations when the infant was placed in an unfamiliar box with different social stimuli. The mother was the most effective in reducing vocalizations, followed by the father, a sibling and an unfamiliar adult female, though statistical analysis was not performed. These results were confirmed by Hennessy and co-workers (Ritchey & Hennessy 1987; Graves & Hennessy 2000; Hennessy et al. 2002). They found that the presence of the mother inhibited plasma cortisol and vocalization responses pre- and post-weaning in guinea pigs that were placed in novel surroundings. The presence of siblings also inhibited these reactions but to a lesser extent, as was the case with an unknown female. On PND 11/12 and 18/19, the anesthetized mother did reduce these reactions either, in contrast with an anesthetized unfamiliar lactating female (Hennessy & Ritchey 1987). Adult males did not suppress the stress reactions. The potentiation phenomenon, in which a brief reunion with the mother prevents normal decline in ultrasonic vocalizations during isolation, is also present in guinea pigs (Hennessy et al., 2006b). Pups on PND10 showed the potentiation phenomenon in reaction to reunion with their mother, but not to the company of a familiar litter mate or an unknown lactating female.
Hennessy et al. (2006a) found that infant-mother interaction reflected adult social organization: in B**razilian guinea pigs** (Cavia aperea), infants living in a harem interacted in a similar way with their mother as with unfamiliar females. In **Galea monasteriensis** living in pairs, infants retained physical closeness with their mother as opposed to other females. Their plasma cortisol levels were also lower in the presence of their mother. Jäckel & Trillmich (2003) let guinea-pig

pups (Cavia porcellus) on PND 5-30 choose between a urine sample from their own mother and a urine sample from another mother. By PND 10, pups were able to distinguish between mother and familiar or unfamiliar and between mother and related or unrelated, but not between mother and lactating females that were both familiar and related.

2.3 Neurohormonal studies in attachment

2.3.A Pharmacological influences on infantile attachment behavior

As mentioned earlier, Shayit & Weller (2001) gave 11 to12-day-old rats a choice between an empty arm and an arm that contained their mother's faeces. The preference for the maternal odor arm increased after administration of selective **CCK-A and CCK-B** antagonists. Nowak et al. (1997), Goursaud & Nowak (2000) and Nowak et al. (2001) could block development of preference for the mother in lambs at 24 hours by giving them a CCK-A receptor antagonist injection at birth.

Ultrasonic vocalizations have been extensively used in tests for anxiolytics and in addiction research (see Sanchez 2003 and Covington & Miczek, 2003, for reviews).

Herman and Panksepp (1978) were the first to report on the relationship between the **opioid** system and attachment behavior. Ultrasonic vocalizations in isolated infant guinea pigs were reduced by morphine and increased by naloxone. The time infants spent in contact with their mother was also reduced by morphine. Morphine also extended the time needed to reach the home cage in a T-maze when juvenile Long Evans were trained to do so (Panksepp and DeEskinazi (1980). The number of trials needed did not change. Extinction of this learned behavior was slowed down by morphine and accelerated by naloxone.

Blass et al. (1990) found that naltrexone did not counteract the quietening influence of maternal odors on USVs in isolated rat pups, while it counteracted the quietening effect of morphine, suggesting that opioid systems do not play a role in the effects of maternal contact. This suggestion was reinforced by Shair et al. (2005), who did not find any effect of morphine or naltrexone on potentiation of USVs (see section on USV potentiation in paragraph 2.A.2). However, Moles et al. (2004) showed that μ receptor knock-out mice did not

react as much with USVs to separation from their mother as did the controls, nor did they show maternal potentiation (see paragraph on USVs in rats). Maternal odors caused a considerable reduction in USVs in wild type, while the knock-out level was so low during isolation that exposure to maternal odors did not make a difference. The implication that the absence of the μ receptor specifically influenced the attachment behavioral system was strengthened by the fact that the knock-out pups also failed to show a preference for their mothers' bedding when given a choice with another dam's bedding. A study by Shayit et al. (2003) was also supportive for a role of endogenous opioids in the development of preference for the mother in lambs, as this preference could be blocked by naltrexone at birth.
Scattoni et al. (2005) lesioned the basal forebrain **cholinergic** neurons with 192 IgG-saporin on PND 7 and measured USVs over the following days, as well as homing behavior on PND 13. They found that the lesion reduced USVs and increased the number of entrances into the nest area, while not significantly increasing the time spent there.
Insel & Winslow (1991) used **oxytocin** (see paragraph 2.C.2 below) to influence the ultrasonic vocalizations that rat pups emit in response to isolation. They found that central administration of 500 and 1000ng of oxytocin reduced the vocalizations and that this effect was blocked by an oxytocin antagonist. The response to peripheral administration of oxytocin was biphasic, depending on the dose. Later studies (Winslow et al. 2000) with oxytocin knock-out mouse pups showed that the knock-outs vocalized less than wild mice during separation from the mother. The same reduction in vocalizations was found in oxytocin-receptor knock-outs (Takayanagi et al., 2005).
The same group (Winslow & Insel 1993a) also centrally administered 500 and 1000ng of **vasopressin** and found that it also reduced vocalizations, the effect of which was blocked by a V1a receptor antagonist. Peripheral administration of 100 and 500ng of vasopressin increased vocalizations, while a V1a and a V2 receptor antagonist could not counteract this effect. A V1b receptor antagonist (Ijima & Chaki, 2005) reduced vocalizations in isolated pups. Hodgson et al. (2007) report that intraperitoneal administration of 30mg/kg of the V1a antagonist SSR 149415 reduced USVs in rat and guinea pig pups.
Nelson & Panksepp (1996) paired exposure to an odor with contact with the mother on PND 14 and measured preference for that odor the following day. The odor was preferred more if exposure had taken place with the mother present than without the mother present. Central

administration of **oxytocin** did not influence the development of this preference, but an oxytocin-antagonist prevented it, while the oxytocin antagonist did not affect single-trial avoidance learning, nor did it disrupt interaction with the anesthetized mother. Winslow et al. (2003) report that oxytocin concentrations in cerebrospinal fluid were reduced in male rhesus monkeys raised in nurseries as opposed to monkeys raised by their mothers.
In young adult pigtail macaques, Rosenblum et al. (2002) found lower concentrations of oxytocin and higher concentrations of **CRF (Corticotropin Releasing Factor)** than in bonnet macaques. This was speculatively attributed to the smaller amount of social affiliation in the former.

2.3.B Neurohypophyseal hormones and partner preference/pair bonding

Oxytocin and vasopressin are nonapeptides, predominantly synthesized in the paraventricular and supraoptic nucleus of the hypothalamus and released in the posterior part of the hypophysis. As yet, there is only one oxytocin receptor present throughout the brain, although the precise distribution is species specific. Peripherally, oxytocin has a role in the physiology of parturition and lactation, promoting contraction of the uterus and mammary glands respectively. Vasopressin has three receptors, the V1a, V1b and V2 receptor. The latter has not been located in the brain, while the first two have been found in numerous parts of the brain and the V1a receptor in particular is present in abundance. Peripherally, vasopressin maintains blood pressure by concentrating urine in the kidneys and constricting blood vessels.
Both hormones play a role in the stress response. While vasopressin stimulates the HPA-axis (Armario, 2006), oxytocin seems to dampen the stress response (Heinrichs & Gaab, 2007). Oxytocin and vasopressin also have a number of behavioral central effects (see Caldwell & Young 2006 for a review). Oxytocin promotes maternal behavior, female and male sexual behavior, female aggression, social recognition, scent marking and grooming and spatial learning in females, while it reduces anxiety and, in some tests, negatively influences memory. Vasopressin promotes aggression, flank marking and social recognition, increases anxiety and generally facilitates learning.

Research on pair bonding in rodents has in recent years largely been performed in two vole species: the Microtus ochrogaster (prairie vole) and the Microtus montanus (montane vole). The prairie vole is a highly social species, in which males and females form monogamous pair bonds following mating and jointly raise their offspring. Montane voles live in isolated burrows, do not form pair bonds, males are not parental and females leave their offspring in the second or third post-natal week. A number of reviews of the work carried out on these animals have been published in the last 15 years (Carter et al., 1995, Insel 1997, Insel & Young 2001, Young & Wang 2004, Lim & Young 2006).
In short, prairie voles require several days of cohabitation and mating to form pair bonds. In female prairie voles, central infusion of oxytocin during cohabitation facilitates the forming of pair bonds, in that mating is no longer required (Williams et al., 1994). Oxytocin antagonists prevent the forming of pair bonds, even when mating occurs. In male prairie voles, vasopressin has the same facilitating role (Winslow et al., 1993b) and the vasopressin antagonist the same blocking effect. Autoradiographic studies show that the distribution of oxytocin (Insel & Shapiro 1992) and vasopressin receptors (Insel et al., 1994) are very different, almost complementary, in monogamous prairie voles and isolated montane voles, while the sensitivity and the DNA of the receptors do not differ. Mice that were transgenic for the prairie vole V1a vasopressin receptor showed a pattern of receptor distribution that was comparable to the prairie vole and reacted with affiliative behavior to the administration of vasopressin (Young et al., 1999). Neuroanatomically, the nucleus accumbens in females (Young et al., 2001) is important because it was possible to facilitate or block development of pair bonds by locally injecting oxytocin or an antagonist. In males, the ventral pallidum (Pitkow et al., 2001) plays a major role, as increasing V1a receptor density through a viral vector in this region resulted in increased pair bonding. In recent years, both dopamine (Aragona et al. 2006) and CRF (Lim et al. 2007) have been found to also play an important role in the development of pair bonds in prairie voles.

2.4 Conclusion

Rodent infants recognize and prefer their mother from very early on. Evidence from rat pups suggests that antenatal olfactorial learning

plays a role in this. Extensive research has shown that all kinds of stimuli can become attractive to the pup in an attachment context, with the degree of attraction depending on the degree of similarity to, or extent of association with, the mother. Yet other stimuli that can provide nourishment or warmth (like other lactating females, paternal figures or cage mates) are also attractive to pups, although less so than the mother. In guinea pigs the attraction generated by different adults is to some extent a reflection of social organization. Whether the development of the attachment to the mother is a consequence of the satisfaction of the physiological needs of the pup, or has an independent neurobiological origin, is not easy to establish in rodents, unlike the situation in monkeys (Harlow 1958). Recognition has clearly been established immediately after birth, before the mother could have become associated with the fulfillment of the physiological needs through a sensory learning process. Yet limited motor abilities in this phase of development preclude the assertion that this recognition is indeed a preference and as such a manifestation of attachment. Nevertheless, a central hypothesis of Bowlby's attachment theory - that there is an innate tendency to form a preferential bond with a specific other, regardless of reinforcement through administration of food or warmth - is not falsified.
Pharmacologically, although there are some difficulties in interpreting results, generally speaking oxytocin facilitates attachment-like behavior. In prairie voles, however, only pair bonding in females is promoted by it. A more detailed description of the way oxytocin works is hard to give. Is oxytocin involved in the switching on of attachment behavior in order to achieve the goal of proximity to the attachment figure? Endorphins might then be the rewarding substance that switches off the behavior when the goal is reached. Another possibility is that oxytocin itself is the rewarding substance, for instance by releasing dopamine. The same lack of evidence with regard to exact mechanisms is present in vasopressin. There the picture is even more complicated, given the contradictory effects of vasopressin and its antagonists on USVs.
In both oxytocin and vasopressin, preference tests in an ecologically valid infant-mother attachment paradigm are lacking.

2.5 Aim and outline of the thesis

This thesis investigates whether the rat can serve as an animal model for research on the neurobiological substrate of attachment behavior, especially infant-mother attachment. Although attachment theory is the most - if not the only – evidence-based theory in developmental psychology, it has not been the framework for many animal studies. The neurobiological substrate of attachment behavior therefore remains to be elucidated. This has been an obstacle for the acceptance of attachment theory in psychiatry (adult psychiatry in particular), particularly because there has been an emphasis on biology in recent decades. If the rat could be used as an animal model, this would have several advantages. Rats can be kept relatively easily, they have large litters allowing within-litter pharmacological comparisons and much is already known about their physiology and behavior. Monkeys and voles that have been used for attachment-related research in the past, are less suitable due to costs, ethical issues and litter size.
In **chapter 1**, the origins of attachment theory are described and the evidence for some of its central postulates is reviewed. In the second part of this chapter, an extensive overview of the relationship between attachment variables and psychiatric disorders is presented, to answer the question as to whether or not attachment theory is relevant for (child) psychiatry.
In **chapter 2**, studies are reviewed that may contribute to the knowledge about attachment-related behavior in rodents, although they may have been performed from different theoretical perspectives, such as sensorimotor development and physiology of the stress response or learning may have been the reason for this research. However, the hope is that these studies can yield information about attachment and its neurobiological substrate if results are interpreted in this context. The pharmacological research will also be presented with a more explicit relation to attachment behavior, such as ultrasonic distress vocalizations in rat pups and partner preference in voles,.
In **chapter 3**, an experimental set-up for research on attachment behavior in rat pups is proposed. It offers the pups an opportunity to choose between two home environments. They each contain a rat mother and her pups, both nests being born on the same day. It examines whether a preference for the own home side is present and how this preference develops in the period before weaning. Some

control experiments are performed to assess whether the preference was influenced by repeated testing.
In **chapter 4,** an improvement in the experimental design used in chapter 2 is introduced. In this set-up, rat pups are offered a choice between four instead of two home environments, one, of course, being the one in which the pup grew up. In the light of the dominance of kin selection theory in ethology, we try to elucidate whether genetic relatedness or familiarity is the more important factor leading to the preference for the home side. To test construct validity of the rat model, the effect of the neuropeptides oxytocin and vasopressin on the preference for the home side is measured.
In **chapter 5,** studies are performed to see if a partner preference could be induced in adult male rats, although in nature their social organization is polygamous. The idea is to simulate the partner preference induction done in prairie vole research. If this is the case, then the presence of a neurobiological substrate for specific attachments in adulthood in rats can be expected. This neurobiological substrate can be activated contingent upon experiential factors or ecological circumstances. A specific factor that may play a role in the development of partner preference is also investigated, namely olfactorial stimuli during the cohabitation period and subsequent recognition of these self-referencing stimuli during testing of the preference.
In **chapter 6**, a pivotal question is addressed: do attachment disturbances during development lead to behavioral disturbances in adulthood? If normal cognitive and emotional development is hampered by the absence of a reliable and caring specific attachment figure, this leads to deficiencies in coping capacities, increased vulnerability to stress and insufficient affectionate relationships in adulthood. As a result, frequency and severity of psychopathology is increased in humans. A detrimental effect of attachment disturbance during infancy on adult rats would strengthen the face and predictive validity of the model. To study this, repeated cross-fostering studies are performed. In the first study, rats are cross-fosterd once a day, in the second study twice a day. Care will be taken to equalize the amount of isolation and handling in cross-fostered and non-cross-fostered groups. In adulthood, play behavior, social behavior, open field, elevated plus maze, shock, prod, bury and sexual behavior tests are performed.
In **chapter 7**, the results presented in preceding chapters are discussed and conclusions are drawn. Special attention is paid to integration of

the results of the experimental chapters with the reviews in the first chapter. Extra attention is given to the relationship with kin selection theory and the evolutionary aspects of attachment.

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CHAPTER 3

Attachment in rat pups, an experimental approach.

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Published in: Neuroscience Research Communications 1998 vol. 23(3): 169-179.

Abstract.
John Bowlby's attachment theory states that attachment behavior has been strengthened throughout evolution as a consequence of its adaptive value. We investigated the presence of attachment-like behavior in rat pups, by offering a choice between the home nest and a same aged other nest. Rat pups showed an increase of preference for the home nest during the second week and the first half of the third week. After that a preference for the other home side developed. Control experiments showed that the results were not due to repeated testing. Possible explanations of the results are discussed in light of renewed criteria for attachment in animal experiments. It is concluded that a primitive and temporary form of attachment in rats is present.

Key words: attachment; Norway rat; homing behavior; ontogeny; olfaction; kin recognition

The quality of the affective relations between young children and their primary caregivers affects vulnerability for psychopathology later in life (Klimidis et al . 1992; Rutter 1995; West et al . 1993). John Bowlby's attachment theory (Bowlby 1984) and contributions by Mary Ainsworth (Ainsworth et al . 1978) have been the most influential in describing these relations and have found empirical support (for a review, see Rutter 1995). The nucleus of the attachment theory is that there is an innate tendency to form an affectionate bond, an attachment, with a specific other (usually the mother), the attachment figure. The related behavior is regulated by the attachment behavioral system. This system has a set-point, that influences the distance from infant to mother and promotes proximity in case of distress or danger. The adaptive value of turning to the specific figure that has shown to be protective and nurturant in the past is clear. The attachment behavioral system interacts with the exploratory behavioral system. Exploration, using the attachment figure as a secure base, can occur if the attachment system is relatively idle.
The foundation of attachment theory in ethology implies that there are similarities in the neurobiological substrate across different species. For practical reasons and because of the existing behavioral, neurophysiological and neuroanatomical knowledge about the Rattus Norvegicus we explore if this species can serve as an animal model. Gunnar (1978) suggested criteria for operationalisation of attachment (see discussion) and mentioned recognition of and preference for the attachment object as crucial. Literature on rats explicitly based on Bowlby's attachment theory or on the operationalisation mentioned above is scarce. There is a load of data concerning experiments in which preference behavior was tested for other reasons, for instance in research on sensory, motor or thermoregulatory development or on proximate elicitors of behavior related to kin selection (Sigling et al ., in preparation). Only a few of these experiments describe choices between home environment or maternal cues and comparable cues from other nests/mothers, with conflicting results. Gregory and Pfaff (1971) reported that rat pups of 13-19 days old did not prefer home litter shavings to other litter's shavings. Experiments by Leon and Moltz (1971) showed that rat pups in an air stream test did not discriminate between their mother's odour and the odour of a strange lactating female. On the other hand, Carr et al . (1979) reported that 12 day old rat pups preferred bedding from the home nest to bedding from a nest of the same age, while 16 day olds had no preference and 20 day olds preferred the strange bedding. We decided to design an

experiment in which the stimuli offered to the pup would be similar to the natural situation without the mother being able to retrieve the pup. Our aim was to see if rat pups distinguished and preferred their home environment and to describe the development of this preference, if present. We hoped to find evidence with regard to the conflicting results in the literature and aimed to develop a test that could reflect manipulation of the neurobiological substrate of the attachment behavioral system. We want to stress that our research is directed at the attachment from the pup to the mother and that we do not want to expand on the literature on early separation and its consequences (Hennessy 1997; Hofer 1996; Kaneko et al . 1994).

GENERAL MATERIALS AND METHODS

Animals

Pregnant Albino Wistar mothers were purchased from the Animal Facility of the University of Utrecht (GDL). They were placed individually in the test cage in the experiment room three days before expected delivery and were kept in a reversed day-night schedule. Litters used in one experiment were born within a 24-hour period. Litters larger than 10 pups were culled to 10 on day 1, smaller nests were left intact. Pups remained with their biological mother throughout the experiment.

Materials

The testcage was made of perspex and consisted of three parts: a central test area and two peripheral parts, each containing a mother and her litter. The three parts were separated by two half round partitionings of metal mesh. The cages were 123 cm in length, the width was 25 cm and the height was 30 cm. A 3 cm layer of sawdust, of which a handful was refreshed each day, covered the floor of the nestparts. The floor of the central part consisted of a removable rubber mat. Two of these cages were placed side by side. Two 25 watt red light bulbs and a 36 Watt fluorescent red ceiling-light were installed. A VHS camcorder recorded the movements of the pups in the two central parts from above. Water bottles and RHM 11/10 chow, produced by Hope Farms Inc. of Woerden The Netherlands allowed for ad lib water and food consumption. Humidity was kept at ± 60% and temperature at ± 21.5 C. Materials were the same in all experiments.

Methods

Testing started at postnatal day 8, after a pilot experiment had shown that younger pups lacked the motor abilities to explore the total test area. At the start of testing pups were placed in the centre, always facing the same direction. Time spent on the home nest side was recorded during 15 min. After each pup, soilings were removed and the rubber mat was wiped clean. Two pups were tested simultaneously, one in each cage. After each test day the cages were rotated 180 degrees, so that the next test day the home nest would be on the opposite side. Pups were tested in random order and marked with ink for identification. Time spent at home nest side was scored from videotape by means of the program 'the Observer', supplied by Noldus Information Technology Inc., Wageningen, the Netherlands. Statistical analysis of all experiments was performed by means of SPSS for Windows.

EXPERIMENT 1: PREFERENCE FOR HOME SIDE ON PND 8-24

Statistics

Because the data were not normally distributed in the first days of testing, a binomial test was performed throughout the experiment. One category was defined as spending more than 50% of time at the home nest side and the other as spending more than 50% at the other side. The test group was compared with a hypothetical control group in which there was a fifty/fifty division over both categories. α was set at 0.05, and a Bonferroni correction for multiple testing resulted in an α of 0.0029.

Results

The results are shown in figure 1. A significant preference (2-tailed $p \leq .0001$) for the home cage was seen on postnatal days 16 to 19, with a maximum on day 17, when mean time spent at home nest side was 642.13 of 900 s. Preference was reversed on day 22, and reached significance on day 24 (2-tailed $p=.0027$) when 350.63 of 900 s were spent at home nest side.

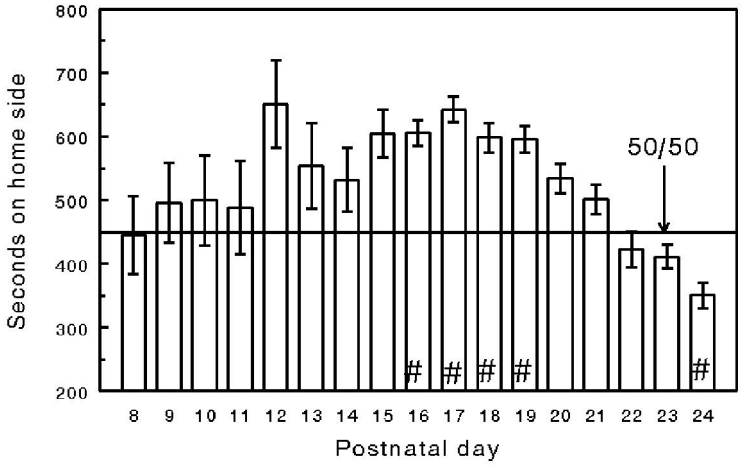

Figure 1. Development of preference for home cage in rat pups. Bars represent seconds spent at home side during 15 min test, ± 1 SEM. # = $p \leq 0.001$.

EXPERIMENT 2: CONTROLLING FOR REPEATED TESTING ON PND 16

To control for learning through repeated testing we performed an experiment in which one group was tested from PND day 8 on and the other only on day 16, near the maximum of the preference for the home side.

Methods

Two nests were tested each day from day 8 on, the other two only at day 16. Testing of the repeatedly tested group continued until day 23. The cages were turned 180 degrees on day 16 after half of both groups had been tested. Therefore, half of each group had the home nest on the right side, the other half on the left side. This procedure was repeated on day 23. Otherwise methods were the same as in experiment 1.

Statistics

The two groups were compared with each other by an independent t-test on day 16. Subsequently they were each compared in a dependent t-test with the hypothetical group in which the time spent on the home

nest side was 450 s. On day 23, when 180 degree rotation was performed after half the pups had been tested, a comparison was made with the 450 s hypothetical group, again by a dependent t-test.

Results

Results are presented in figure 2. The repeatedly tested group ($t(1,19) = 4.38$, $p \leq 0.001$) differed from the hypothetical 450s group as did the once only group ($t(1,15) = 2.67$, $p < 0.02$). Technical difficulties and procedural errors and equalisation of the number of pups from each nest led to a group size of 14 pups each in the comparison between the repeatedly tested group and the once only group on day 16. Although the repeatedly tested sample had a higher mean than the once-only sample, the difference was not significant: $t(1,26) = -.52$, $p= .608$. The preference for the not-home side on day 23 was significant: $t(1,14) = -3,17$, $p= 0.007$.

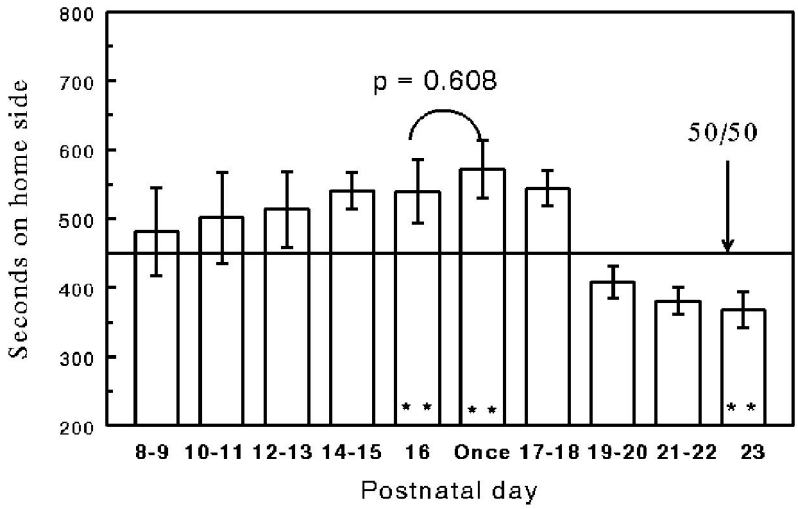

Figure 2. Comparison on postnatal day 16 between pups that were tested from PND 8 on and pups that were tested only on day 16. Bars represent seconds spent at home side in 15 min test, ± 1 SEM. ** = $p< 0.01$.

EXPERIMENT 3: CONTROLLING FOR REPEATED TESTING AFTER PND 19.

This experiment was performed to control for repeated testing effects on the preference for the not-home side after day 19. One group was tested from day 8 on and we planned to test the other after a significant preference for the non-home side had developed.

Methods

Scoring was now done automatically, using Ethovision ®, also supplied by Noldus Information Technology Inc. Diagonal location of same group nests and 180 degrees rotation after each test day was done to control for preference for one side of the room. We had planned to test the once-only group the day after the repeated group had shown a significant preference for the non-home side. But the preference for the non-home side did not reach significance, so on day 25 we decided not to wait any longer and test the once-only group. Again the cages were turned 180 degrees after half of the groups had been tested on day 25. Methods were otherwise the same as in experiment 1.

Statistics

The repeatedly tested group and the once-only group were compared on day 25 through an independent t-test, while both were compared with a hypothetical 450 s group through a dependent t-test.

Results.

The preference for the not-home side did not reach significance in the repeatedly tested group, $t(1,18) = 1.15$, $p = 0.265$ (see figure 3). The difference between the repeatedly tested group and the once-only group on day 25 was significant: $t(1,37)= 2.34$, $p < 0.03$. The preference of the once-only group for the not-home side was significant: $t(1,19) = 2.34$, $p < 0.03$.

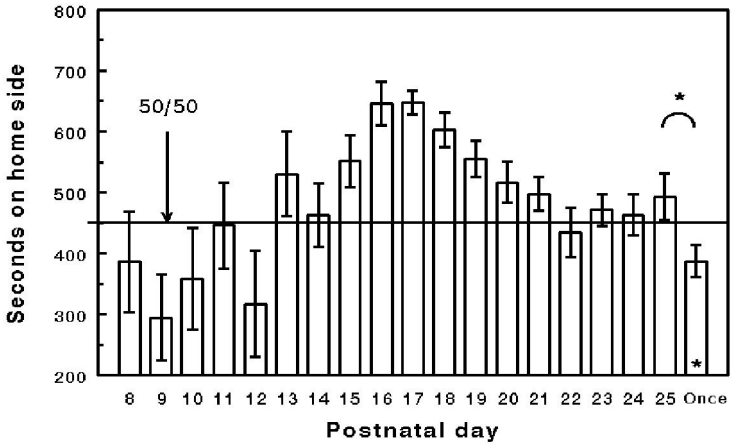

Figure 3. Comparison of preference for home side between pups tested from PND 8 on and pups tested only on day 25. Bars represent seconds spent at home side during 15 min test, ± 1 SEM. * = $p < .05$.

GENERAL RESULTS

To give an impression of the influence of littervariance, we present two figures that show means of the seconds spent on the home side for all the repeatedly tested litters. Figure 4 shows the results on day 16, figure 5 the results of day 23.

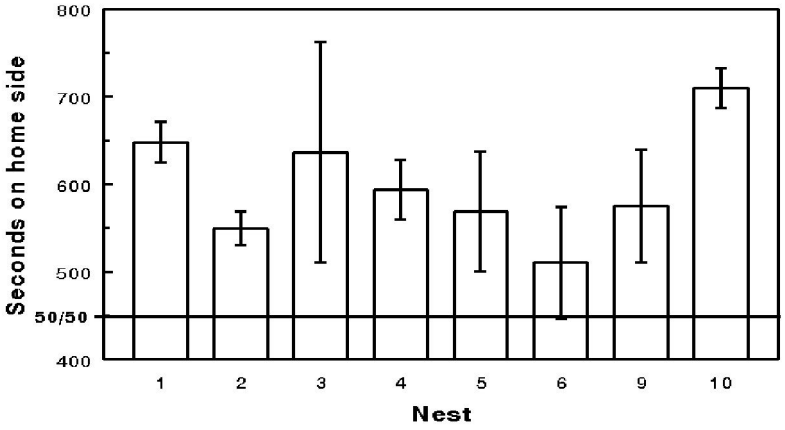

Figure 4. Seconds spent on home side per litter on day 16. Nest 3 contains only 4 animals. Only repeatedly tested litters are included. Error bars represent SEM's.

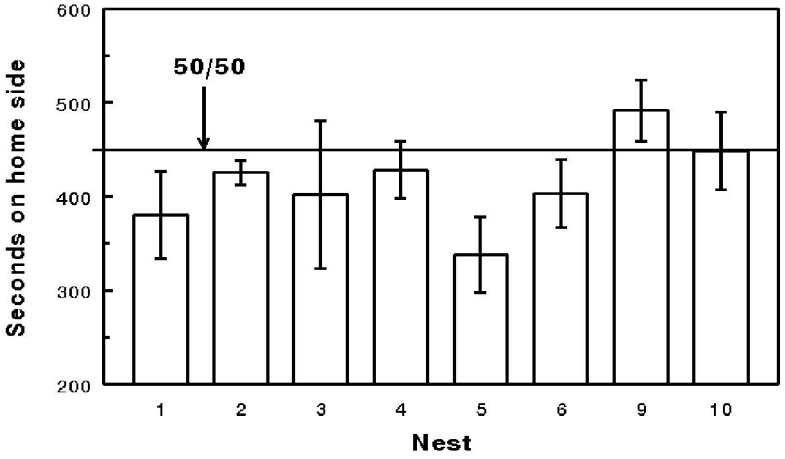

Figure 5. Seconds spent on home side per litter on day 23. Nest 3 contains only 4 animals. Only repeatedly tested litters are included. Error bars represent SEM's.

DISCUSSION.

Rat pups show a biphasic pattern in preference for home in our experimental design. Carr et al . (1979) found the same pattern we did, but our results are not in agreement with the work of Gregory and Pfaff (1971) and of Leon and Moltz (1971) in which rat pups did not distinguish between their mother and a strange lactating female. Carr et al . offered the pups bedding to sniff at and thus provided the same proximity to the stimulus material we did. As Leon and Moltz placed the stimulus animals in an air stream close contact may be necessary for distinction. The lack of significant result as reported by Gregory and Pfaff could be due to the mixed age-group they used (PND13-19) and/or a type 2 error, as the figure in their article showed a non significant ± 65% to 35% preference for home litter shavings over other litter shavings.

Observation during testing revealed that the dams had no influence on the preference of the pups. There was either little and indiscriminate (second week) or no interest at all (third and fourth week) for the test pups. The test results seem to be the consequence of behavior of the pups, not of the dams.

In the third week the preference for the home nest side peaks. The crucial question is if this can be considered attachment in the sense of Bowlby's theory. An alternative explanation could be that it is an aversion for the other side, i.e. neophobia. This is unlikely, since Leon and Moltz (1971) showed that pups prefer a strange mother versus an empty box or a nulliparous female. Also neophobia is considered to develop after weaning (e.g. Bronstein & Hirsch 1976).

A more serious criticism of an attachment interpretation of our results could be that the preference does not concern the mother. Gunnar (1978), drawing from Ainsworth (1972), formulated criteria for an animal model of attachment: 1. The ability of the young to discriminate and respond differentially to the object of attachment; 2. Preference for the attachment figure and differential proximity seeking; 3. Response to removal of the attachment object, which is distinct from responses to the reduction of social stimuli per se. These criteria are somewhat tautological, because it is hard to imagine preference and differential proximity seeking without the ability to discriminate and respond differentially. Yet, they are in accordance with Bowlby’s theory. Our setup does not show if pups prefer their mother, their siblings, the sawdust or a combination of these. This question is partially answered through an experiment by Leon (1974)

where 16 day old showed, in an air-flow discrimination test between the mother and 5 of their own littermates, a 93 % preference for the mother. Further research could answer the question more fully, but runs the risk of confusing proximate and ultimate mechanisms. The adaptive value lies in the attachment to the mother, although all kinds of stimuli -if associated routinely with the mother in her reinforcement of innate attachment behavior- can be directional for this behavior when the infant is in a state of distress. Taking into account the difference between proximate and ultimate mechanisms we propose the following criteria for an animal model of attachment, based on those of Gunnar: 1. Preference for the putative attachment object and/or stimuli that have become associated with this object through reinforcement of attachment behavior; 2. Response to removal of the attachment object which is distinct from the response to reduction of social stimuli per se. Nevertheless, the specificity of the object-attachment in the strict sense according to the criteria is not demonstrated in our experiments.

Another alternative explanation could be that the preference is a kin recognition phenomenon (see Hepper 1986 and Holmes 1988 for reviews). The kin recognition literature as we know it, is flawed by an absence of discussing data in light of attachment theory. Many experiments show no more than exposure or associative learning and no preference for genetically related unfamiliar conspecifics. The conclusion that kin selection does not play a role in these instances is then said to be unjustified because this would be a confusion of proximate and ultimate mechanisms (e.g. Holmes 1988). In this reasoning the proximate mechanisms of exposure learning and associative learning are related to the ultimate mechanism of kin selection, excluding the ultimate mechanism of attachment. Monopolisation of these types of learning by kin selection theory, which has as its hallmark the attention for genetic factors, is not logical. We think changes in the set-point of the attachment behavioral system in its interaction with the exploratory behavioral system account best for our results. The preference for home is most needed when the pup is motorically able to stray away from home but depends on its mother for nurturance. Kin selection theories lack a developmental or a systems perspective and cannot as such explain increases or decreases.

The lack of a significant preference for the non home side in the fourth week in the repeatedly tested group in our third experiment may be due to between-litter variance. One could argue that pups from

one litter should not be treated as independent observations (Holson & Pearce 1992), as their common genetic and environmental background increases the risk of a Type I error. In our experiments there is either a comparison with a constant or the hypothesis tested concerns the similarity between groups, reducing the risk of a Type 1 error. By contrast, our hypothesis that the once only and repeatedly tested pups are similar runs the risk of being falsely rejected through a Type II error. A split-litter technique would on the one side reduce this risk, but ceiling or bottom effects of the accompanying daily handling of all nests would on the other side increase it. Our procedure presumably had its drawbacks in experiment 3. The general results (fig. 5) shows that litters 9 and 10, the two that were repeatedly tested in experiment 3, have the highest preferences for home in week 4 of all. Although this suggests a type II error, the true influence of repeated testing in this period remains as yet unclear.

The prevailing view in literature is that attachment in rats does not exist (Hennessy 1997; Crnic et al . 1982; Kraemer 1992; Mendoza et al . 1980), although research according to sharply defined criteria is scarce. We have shown a period of preference for the home cage and made it plausible that the preference concerns the mother. In future work, we will provide data with regard to the effect of the removal of the attachment figure (Sigling et al ., in preparation). For now, we assert that the existence of attachment in rat pups is not a closed case and deserves further research.

Furthermore, the attempt to create an experimental setup for manipulation of the expression of attachment in rat pups seems successful. It has more ecological validity then previously used preference tests and the preference on days 16-18 is consistent in all experiments. The 70-80 % of time spent on the home side can be increased as well as decreased. This provides an opportunity for research into the neurobiological substrate of attachment behavior in rats. The importance lies in the probable evolution of this substrate from the rat to man and the role attachment problems play in human psychopathology.

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Chapter 4

Home seeking behavior in rat pups: attachment vs. kin selection, oxytocin vs. vasopressin.

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Accepted by the European Journal of Pharmacology.

Abstract.
We are interested in the rat as an animal model for infant-mother attachment. In the first experiment we tried to distinguish between a preference for familiar animals (attachment theory) and a preference for genetically related animals (kin selection theory) with the use of an early cross-fostering procedure. Genetic relationships did not influence preferences in cross-fostered pups on postnatal day17, only familiarity did. Subsequently we investigated if peptides known to influence affiliative behaviors were also effective in the present paradigm. Injection of oxytocin into the cisterna magna did not yield significant effects on preference, while vasopressin and desglycinamide-[Arg8]vasopressin reduced the preference in a dose dependent manner. The effect of vasopressin was completely blocked by pretreatment with the V1a antagonist $d(CH_2)_5Tyr(Me)^2,Arg^8$-vasopressin. We discuss the explanatory power of attachment theory and kin selection theory with regard to preference experiments in rats and the usefulness of the rat as an animal model for infant-mother attachment.

Key words: attachment; Norway rat; homing behavior; sex differences; kin recognition; oxytocin; vasopressin.

1. Introduction

Intensive mother-offspring interactions are widely present among vertebrates, especially among mammals. It is assumed that a genetically programmed behavioral system (Bowlby, 1984) predisposes a young animal to develop a preference for a specific caregiver. These infants display attachment behavior towards the caregiver, especially when in distress. The adaptive value lies in both nutritional and protective advantages. In previous experiments (Sigling et al ., 1998) we offered Wistar rat pups a choice between two nests, each containing a mother and her litter: one the home environment, the other an unrelated nest of identical postnatal age. The results showed a gradual increase in the preference for the home nest from postnatal day (PND) 12 until maximum preference was reached at PND's 16 and 17. Thereafter, a decrease was observed, resulting in a preference for the non-home side on PND 23 in 3 out of 4 experiments.

In the present study we further explore the preference of pups for the home zone in a two way and in a four way choice experimental setup. We address the question if the preference around PND 18 is the result of attachment to familiar animals (according to attachment theory) or to genetically related animals (according to kin selection theory, Hamilton 1964). Kin selection theory explains behavior that is detrimental to individual survival by introducing the concept of 'inclusive fitness'. This means that in some situations animals can favor the survival of (part of) their own genes through altruistic behavior directed towards kin. Another advantage of recognition of kin could be optimizing the balance between inbreeding and out breeding.

According to attachment theory, pups have a genetically programmed inclination to specifically develop a preference for those stimuli that are present during reinforcement of attachment behavior. The ultimate difference between attachment in Bowlby's sense and kin selection in Hamilton's sense is clear: attachment increases the survival of the individual and hence of the individual's own genes; kin selection derives its biological relevance from explaining behavior that at first sight could seem disadvantageous to the individual but ultimately has selective advantages for (a part) of its genes.

The nature of the social behavior of rats makes them a good object for the study of the supposed benefits of kin selection. They live in groups with multiple paternity, a situation in which the selective advantages of nepotistic behavior towards kin and those of optimal out breeding

are evident. This, combined with the existing wealth of knowledge on the physiology and other behaviors of the rat should make it an interesting animal model to study recognition of genetically related animals.
For the pharmacological experiments, we focused on oxytocin and vasopressin. Reports on the role of oxytocin and vasopressin in social behavior in different species in general and pair bonding in particular (Insel, 1992; Ferguson et al ., 2002; see reviews by Lim and Young, 2006 and Carter et al ., 2008) inspired us to focus on these two nonapeptides. They are predominantly synthesized in the paraventricular and supraoptic nucleus of the hypothalamus and released in the posterior part of the hypophysis (Brownstein et al . 1980). Oxytocin has been shown to affect sexual, maternal, social and stress-related behavior and to play a role in feeding, grooming and learning (see Gimpl and Fahrenholz, 2001 for a review). In general, oxytocin has been characterized as the hormone of affiliation (Insel, 1992). Vasopressin maintains blood pressure by concentrating urine in the kidneys and constricting blood vessels and it promotes aggression, flank marking and social recognition, increases anxiety and generally facilitates learning (see Caldwell and Young, 2006 for a review). Both peptides play a role in the stress response. While vasopressin stimulates the HPA-axis (Armario, 2006), oxytocin is reported to dampen the stress response (Heinrichs and Gaab, 2007).
In experiment 1 pups were cross-fostered immediately after birth to another nest and the influence of genetic relatedness and familiarity on the preference for different environments was investigated. In experiment 2, the influence of oxytocin, injected into the cisterna magna, on the preference for the home side was studied. In experiments 3, 4 and 5 the influence of vasopressinon the preference for the home side was characterized.

2. Materials and methods

2.a. Animals

Pregnant out bred U:WU(CPB) Albino Wistar female rats were purchased from the Central Animal Facility of Utrecht University. They arrived in our laboratory three days before expected delivery and were kept in separate polycarbonate cages on a reversed day-night schedule -lights off at 7.30 am, lights on at 7.30 pm- from then

onwards. Water and lab chow were present at lib. Humidity was kept at circa 60% and temperature at circa 21.5 C. Day of birth was defined as Postnatal Day (PND) 0.
As our experimental setup allowed for testing of 4 litters on one day, 5 pregnant females were ordered per experimental day. The 4 litters that contained the most pups were selected. Pups were only used if at least one same sex littermate was present, this to ensure within-litter comparisons. In some of the pharmacological experiments pups were only used if at least two same sex littermates were present. Pups without a same sex littermate remained in the nest. Sawdust was replaced twice weekly, until placement in the test cage. Pups remained with their biological mother and nest mates throughout the experimental period. All pups were used only once.

2.b. Apparatus

Two test cages were used. The first was identical to the one described before (Sigling et al . 1998), and had two arms on either side of a central test area, one containing the home nest of the test pup. The second (see figure 1) was a modification of the first one and contained four arms instead of two, again with one arm containing the home nest. It was developed to increase the difference between chance and choice preference, by decreasing the time spent by chance to 25%.

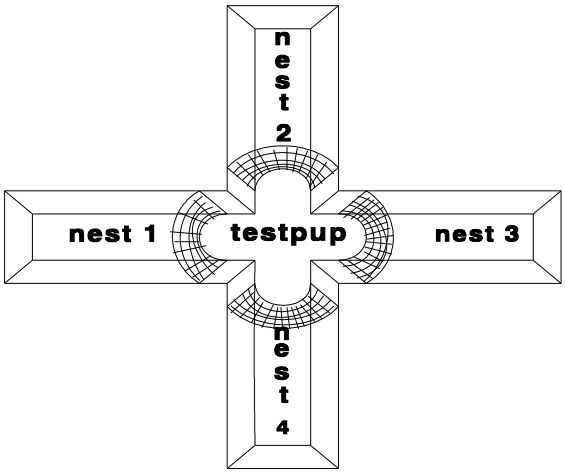

Figure 1. Test cage as seen from above.

Development of preference in the four way choice cage mirrored the development in the two way choice: a gradual increase from PND 12 until PND 17 & 18, with a decline thereafter (data not shown). Both cages were made of polycarbonate.

The peripheral parts containing the litters were separated from the central area by half round partitionings of metal mesh.
The cages were 123 cm in length, the width was 25 cm and the height was 30 cm. A two cm layer of sawdust covered the floor of the nest parts. The floor of the central part consisted of removable black cardboard. Lighting was provided by a 36 Watt fluorescent red ceiling-light. A VHS camcorder recorded the movements of the pup in the central part and the images were processed by a computer with Ethovision software (Noldus Information Technology, Wageningen, the Netherlands). Measures of time spent in the zone near the home nest and latency to that zone were enabled by digitally dividing the image of the central test arena in two zones in the two way choice setup and in four zones in the four way setup.

2.c.1. Experimental design (Experiment 1)

As soon as possible after birth, half the number of pups of each litter were exchanged with pups from other litters, and half remained with their biological mothers. Pups were marked on PND 0 by ink tattoos in the soles of the hind feet. All exchanges were made within six hours after birth and litters were then culled to eight pups. Eight litters remained, each containing 4 male and 4 female rats, 2 non-fostered and 2 fostered. A maximum of two cross-fostered pups originated from the same nest. The day before testing the dams and their litters were marked on their tails with a felt tip pen and then placed in one of the four arms of the four way choice experimental setup that was used in this experiment. The four firstborn litters were tested on one day, the four lastborn the next, resulting in all pups being tested on PND 17. Pups were tested in the following order: A same-sexed pair from one nest, one fostered, the other non-fostered (in random order), followed by a non-fostered/fostered pair of the other sex from the next nest, a non-fostered/fostered pair from this second sex from the third nest, a pair from the first sex from the 4th nest and so on. Fifteen min prior to testing pups were placed in a standard small lab cage of polycarbonate filled with a layer of approximately 2 cm of fresh sawdust. At the onset of testing pups were placed in the middle of the

test arena, always facing nest 2 (see figure 1). Time spent in the home nest area was recorded during 15 min. Total distance moved during the 15 min was used as an activity measure. After each trial , the soiling was removed and the bottom was wiped clean with a paper towel.

2.c.2. Experimental design (Experiment 2&3)

These were peptide dose-finding experiments in the two way choice setup that for practical purposes were subdivided in two parts, as the number of same-sex pups in one litter was a limiting factor. Pups were 17 days old when tested. In experiment two, pups were injected in part one with saline, 1 ng or 10 ng of oxytocin, (supplied by Organon, Oss, The Netherlands) and in part two with saline, 0.1 μg or 1 μg oxytocin. In experiment three, pups were injected in part one with saline, 1 ng or 10 ng of vasopressin, in part two with saline, 0.1 μg or 1 μg vasopressin. (Arginin)Vasopressin-1-9[Arg]^{8} was supplied by Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands. Volume of injection was 10 μl. Pups were injected in the cisterna magna, 30 min prior to testing. After the injection the pups were kept isolated until testing in a small laboratory cage with a layer of 2 cm of fresh sawdust. At the start of testing, consecutively all pups were put in the test arena with their head in the same direction. Test duration was 15 min, after which pups were returned to their home cage.

2.c.3 Experimental design (Experiment 4)

In this experiment pups were injected on PND 17 in the cisterna magna with 30 ng Desglycinamide-[Arg8]vasopressin (DGAVP, supplied by Organon, Oss, The Netherlands) or saline. The experiment was performed to control for possible peripheral effects of vasopressin in the previous experiment. DGAVP has little or no peripheral effects and is thought to have a central effect three times less strong than vasopressin (Gaffori & de Wied 1985). As in the previous experiment 10 ng vasopressin appeared to have the greatest effect on the preference for the home side, without influencing the traveled distance, DGAVP was administered at a dose of 30 ng. Otherwise the procedure was identical to experiments 2 and 3.

2.c.4. Experimental design (Experiment 5).
Pups were 17 or 18 days old. They were injected subcutaneously 45 min before testing with 1 μg of the vasopressin 1a-antagonist $d(CH_2)_5Tyr(Me)^2,Arg^8$-VP (Nambi et al . 1986) in 0.2 ml saline or saline alone. The V1a-antagonist was a gift of Dr. M. Manning, Medical College of Ohio, Toledo, Ohio. 30 Min before testing pups received a subcutaneous injection of 1 μg (Arginin)Vasopressin-1-9$[Arg]^8$ in 0.2 ml saline or saline alone and were kept isolated from then until testing. Subcutaneous injection was chosen because two injections in the cisterna magna within a short period of time was considered too stressful. Dose of vasopressin and antagonist were based on the results of experiment 2. There, the most effective dose of vasopressin that did not influence traveled distance was 10 ng. This dose was multiplied by a factor 100 to compensate for dilution when given subcutaneously instead of intracranially. Otherwise methods were the same as in the previous experiments.

2.d.1.Statistical analysis (Experiment 1)
Two comparisons were made. First, the difference in preference for the home zone between fostered and non-fostered pups was measured. A two-way ANOVA was used, with fostered or not and sex as a factor and time spent in the home zone as dependent variable. Weight was entered as a covariate. Second, only in the fostered group the time spent in two non-familiar zonetypes was compared: the zone of the genetic mother and the combined time of the two other (non related and non familiar) zones divided by two. This was performed with an ANOVAR with zone type as Within-subject variable, sex as Between-subject variable and weight as a covariate.

2.d.2. Statistical analysis (Experiment 2,3,4 & 5).
Time spent in the home zone and latency to reaching this home zone were chosen as dependent variables representing the preference for the home side and ANOVA's were performed for analysis. Following a Bonferroni correction, α was set at .025. Peptide dose and sex were the independent factors. Traveled distance was analyzed separately.

Results

3.1. Experiment 1. Cross-fostering: influence of genetic relatedness and familiarity on preference for different zones.

Non fostered pups did not have a greater preference for the zone they grew up in (H+G+) than fostered pups (H+G-, n=62, F=1.290, p= .261), see fig. 2. There were no significant sex differences (F=0.497, p=.494). There was no significant interaction between foster status and sex (F=1.624, p=.208).

In the second comparison, in which only fostered animals were used, the mean time fostered pups spent in the unfamiliar zone of the genetic mother (H-G+) was not greater than the mean time spent in the two non familiar unrelated zones (H-G-), n=31, F=1.77, p=.194. In fact, fig. 2 shows that there was a nonsignificant preference for the unfamiliar, unrelated zone (H-G-) in both sexes. There was a sex difference in that fostered females had a greater preference for the genetically unrelated unfamiliar zone (H-G-) above the genetically related unfamiliar zone (H-G+) than fostered males (n=31, F=6.56, p=.016), see fig. 2. The interaction between sex and time spent in the zones was nearly significant: F=3.85, p=.06.

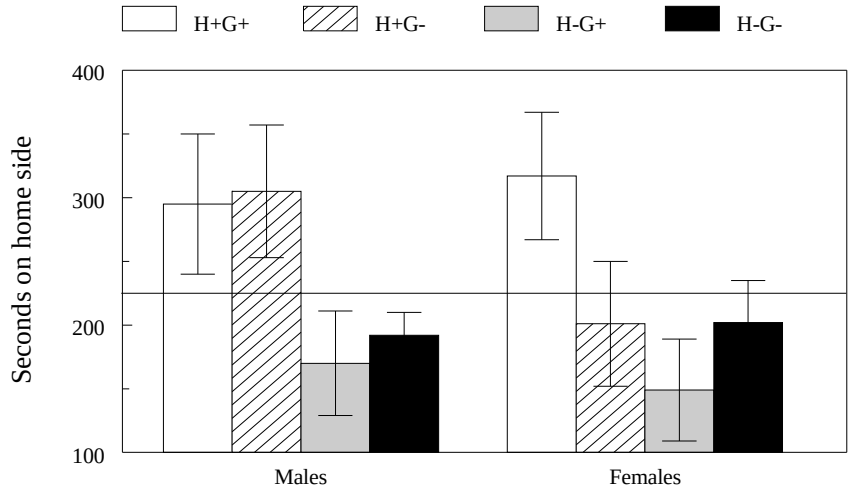

Figure 2. Time spent in different zones during 15 min preference test on postnatal day 17. H=zone of growing up, G=zone containing biological mother and siblings. Straight line is number of seconds expected by chance. N= 8 animals per group on average. Error bars represent SEM's.

3.2. Experiment 2. Dose finding study of oxytocin in two way choice preference test.

In the low dose comparison (saline, 1 and 10 ng oxytocin) the ANOVA's with seconds spent at home side and latency to the home side as dependent factors did not reveal a significant effect of oxytocin (n=75, F=.272, p=.736 and F=.455, p=.636). The higher dose comparison (saline, 0.1 and 1 μg oxytocin) was also not significant (n=63, F=.869, p=.414 and F=1.565, p=.218 respectively). Sex was not a significant factor, there were no significant interactions between sex and oxytocin and travelled distance was not influenced by sex or oxytocin, regardless of dose. Post-hoc analyses of different groupings of the dosages were not significant.

3.3. Experiment 3. Dose finding study of AVP.

The ANOVA of the low-dose comparison (saline, 1 and 10 ng vasopressin in the cisterna magna) did not yield significant results (n=112, F=2.095, p=.128 for seconds on home side, F=.799, p=.452 for latency to home zone). There were no significant sex differences, and the interaction between sex and vasopressin was not significant. Travelled distance did not differ between groups.
In the high-dose comparison (saline, 0.1 and 1 mg vasopressin in the cisterna magna) the latency for the home zone was significantly affected by vasopressin dose (F=3,997, p=.022), while the time spent in the home zone was not (F=2.403, p=.095). See fig. 3 for a combination of low and high dose comparison in latency to home side. Travelled distance was influenced by vasopressin dose (F=27.371, p=.000), see fig. 4.

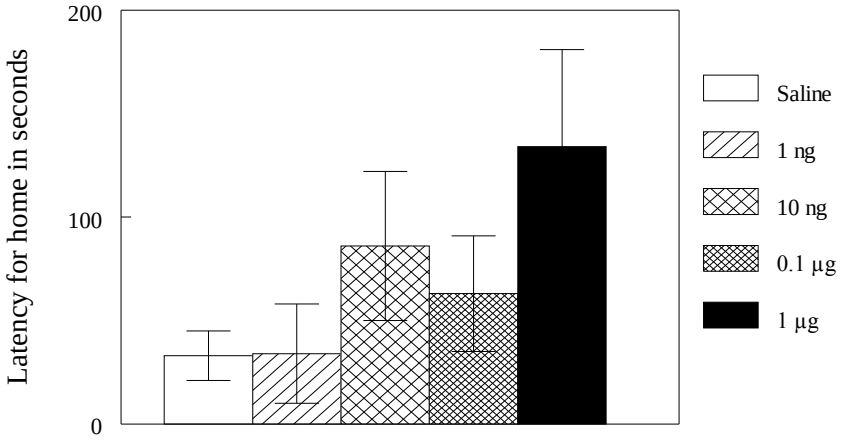

Figure 3. Influence of different doses of vasopressin on latency to reaching home side in a choice between two cages. 15 Min test on PND 17, injection in cisterna magna. Error bars represent SEM's. N=38 in saline, 19 on average in other groups.

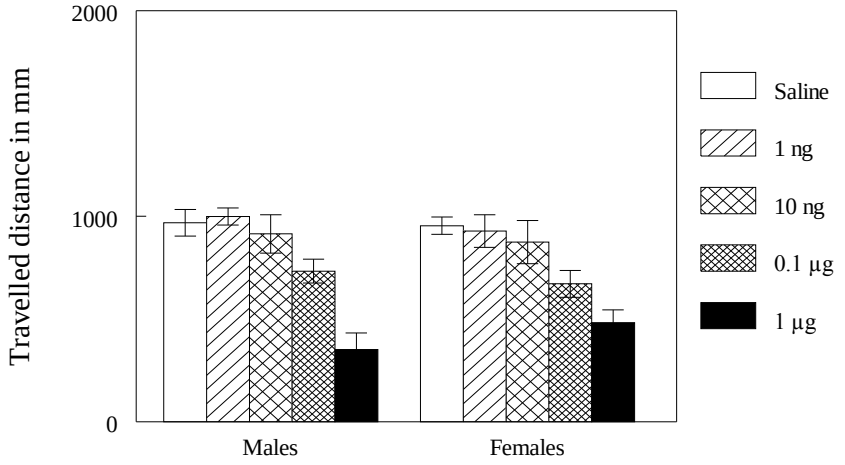

Figure 4. Influence of vasopressin dose on travelled distance. 15 Min test on PND 17, injection in cisterna magna. Error bars represent SEM's. N=38 in saline, 19 on average in other groups.

In a post-hoc analysis, the low end of the dose range (saline and 1 ng of vasopressin) was compared with the dosages of 10, 100 and 1000 ng. Both latency to home side (F=5.897, p=.016) and time spent in home zone (F=5.147, p=.024) were significantly influenced by vasopressin. Sex was not a significant factor and there were no significant interactions between sex and vasopressin.
To statistically validate the preference for the dose of 10 ng yielded by fig. 3 and 4, a second post-hoc analysis was performed in which all dosages were individually compared with saline. The 10 ng dose was the only one that showed a p value of less than .1 in both preference measures while not significantly influencing the travelled distance (F=.919, p=.340).

3.4 Experiment 4. Influence of DGAVP on preference for home side.

Time spent on the home side was significantly reduced (F 5.496, p=.022), see fig. 5, while latency to home side was not (F=.538, p=.465). Sex was not a significant factor and there was no significant interaction between DGAVP and sex. DGAVP did not have a significant effect on the travelled distance.

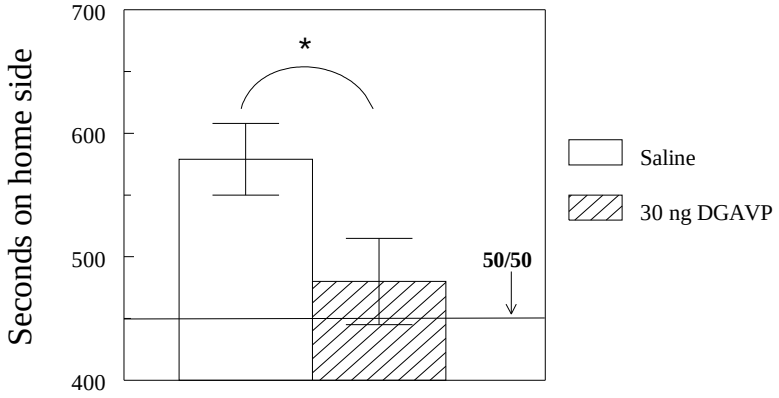

Figure 5. Influence of 30 ng DGAVP on time spent on the home side in two way choice preference test. 15 Min test on PND 17, injection in cisterna magna. Error bars represent SEM's. Horizontal line indicates value expected by chance. * = p < .05. N=41 per group.

3.4. Experiment 4. Influence of vasopressin on the preference for the home side.

The seconds spent at home side were not influenced by vasopressin (n=148, F=.283, p=.596), the V1a-antagonist (F=.035, p=.851 or sex (F=.642, p=.424) nor was the latency to the home side (vasopressin F=2.078, p=.152, V1a-antagonist F=.007, p=.932, sex F=1.794, p=.183). Two way interactions were not significant. The interaction between all three independent variables however was significant in the latency to home side (F=4.931, p=.028). For that reason, sexes were then analyzed separately. In male pups, both vasopressin (n=68, F= .080, p=.778) and the antagonist (F= .323, p=.572) did neither have a significant influence on seconds spent at home side, nor on latency to home side (vasopressin F=.025, p=.875, antagonist F=.130, p=.720). There were no significant interactions.
In females however, vasopressin increased the latency for reaching the home zone significantly (F= 5.427, p=.022), see fig. 6, while there was no effect on time spent in the home zone (F=.232, p=.632). The V1a antagonist did not have a significant effect (F=.140, p=.709 for seconds on home side, F=.161, p=.689 for latency). The interaction between the two was not significant. Travelled distance was not significantly influenced in any of the analyses.

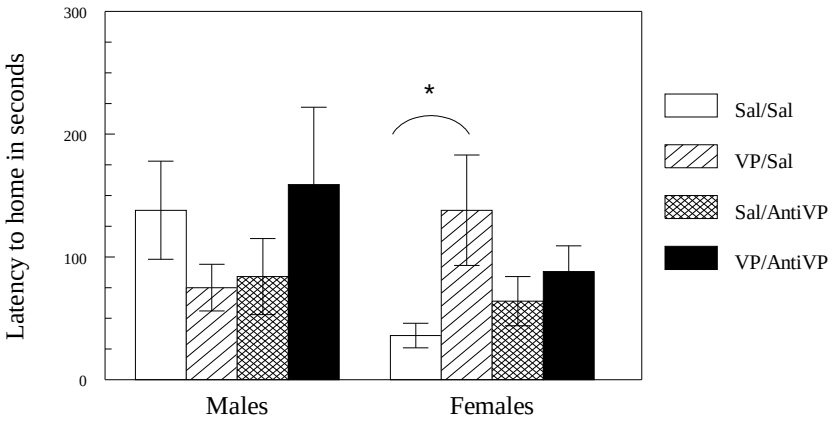

Figure 6. Influence of vasopressin and V1-antagonist (both injected s.c.) on latency to reach home side. 15 min 4 way test on PND 17 & 18. Error bars represent SEM's. * = p <.05. N= 18-19 per group.

4. Discussion.

The first experiment addresses the underlying mechanism that is responsible for the behavior in the preference experiments. Both attachment theory (Bowlby, 1958) and kin selection theory (Hamilton, 1964) have potential relevance. In the context of kin selection theory the preference is presumed to be the consequence of kin recognition. Hauber and Sherman (2001) discuss the three stages of the kin recognition process: production of signals, perception (kin recognition sensu stricto) and the action component. Several authors suggest that there is no specific perception mechanism that recognizes certain stimuli as coming from genetically related animals (Tang-Martinez, 2001; Porter, 1988). They assume that kin recognition sensu stricto is a consequence of association learning: the preference (i.e. the action component) is a result of a match between the encountered stimuli and the content of olfactory memory. Olfactory stimuli stored in memory in infants are usually the result of interactions with genetically related conspecifics or even with the olfactorial stimuli emanating from the recognizing animal itself (self-referent phenotype matching, see Hauber and Sherman, 2001 for a review). That familiar stimuli, leading to preference behavior, stem from genetically related individuals does not imply that these individuals are recognized or preferred because of the genetic relationship. Still, it would be hard to envision selective advantages along the lines of kin selection theory in the natural social context of the rat if there is no differential behavior toward kin.

In our experiment, there were no significant differences in preference behavior as a result of genetic relatedness. As shown in fig. 2, in male pups the situation is clear: preference as a result of familiarity, no effect of genetic relatedness. In females, the preference for the familiar was absent in the absence of genetic relatedness, while in the choice between the two unfamiliar groups there was even a nonsignificant preference for the unrelated nests. This implies that while in experiments with limitation of stimuli rats may be able to discriminate all kinds of genetic relatedness (see Hepper, 1983 and Hepper, 1991), in a more ecologically valid setup this does not have behavioral consequences. A limitation of our experiment was that the two unfamiliar, unrelated nests each contained one or two pups that was a sibling of the test pup. We assume that their contribution to the stimulus properties of the nest were very small and certainly much smaller than the contribution of the dam and the remaining 4 siblings

in the genetic related unfamiliar nest but there is no absolute certainty that there was no influence. However, from the results in female pups this influence seems highly improbable.
With regard to a developmental perspective: Generally speaking, before weaning the familiar is preferred and after weaning the unfamiliar, or there is no preference anymore (Carr et al ., 1979; Hepper, 1983; Hepper, 1986; Sigling et al ., 1998; Wills et al ., 1983; Kruczek, 2007). Attachment theory offers, with its interplay between attachment and exploration behavioral systems, an explanation for this developmental change. We do not know of any explanation according to kin selection theory. Nor are we aware of any explanation about the adaptive value of the preference of a pup for its mother or a familiar environment based on kin selection theory. On the contrary, it could be speculatively argued that a preference for a different dam or environment leaves more maternal resources available for siblings. This confirms that the point made by Blaustein et al . in 1991 is still actual: there is a general lack of knowledge about the adaptive value of kin recognition in vertebrates.
The lack of a significant effect of oxytocin is disappointing, considering the importance oxytocin has in the field of research on affiliative behaviors (Carter et al . 2008). Pilot experiments in our lab with an oxytocin antagonist given intracranially and with subcutaneous oxytocin (data not shown) revealed a trend towards an increase of preference for the home nest side in both. A reason for these unpromising results may be that attachment behavior is strongly connected with learning, with the stress response and with motivation for affiliative behavior. In all three, oxytocin plays a major role (Gimpl & Fahrenholz 2001). Potentially different effects of different concentrations in different areas of the brain on these three constituents of attachment behavior present a confusing picture. At the moment, in the absence of a specific direction, we do not think it is useful to pursue the role of oxytocin in these type of preference experiments further.
The consistent results with vasopressin however are more promising. In the dose finding study it could be argued that the result was partly influenced by aspecific peripheral effects of vasopressin. The DGAVP experiment however, showed that the effect was central in nature and not dependent for instance on motor inhibition. This was confirmed in the combined experiment with vasopressin and the V1a antagonist, albeit only in female pups. The literature on some of the putative components of attachment behavior suggests an increase by

vasopressin. Vasopressin is important in social motivation and social recognition in rats and mice and it stimulates the HPA-axis (Lim and Young, 2006; Bielsky et al ., 2004; Egashira et al ., 2007). An increase in stress theoretically increases attachment behavior. In our, more specifically attachment oriented, paradigm vasopressin decreases attachment behavior. This implies that attachment behavior is more than the sum of its putative parts.

Other research more specifically related to attachment is performed in voles. Prairie and montane voles differ in social organization (see reviews by Lim and Young, 2006 and Carter et al ., 2008). The prairie vole shows monogamy and biparental care (Ruscio et al ., 2008), while the montane vole does not. The literature is unclear with regard to the effects of vasopressin on pair bonding. Although vasopressin in male prairie voles promotes partner preference (i.e. specificity in attachment behavior, Lim and Young, 2004), the results in female voles are contradictory (Insel and Hulihan, 1995, Cho et al ., 1999). The few results in rat pups in ultrasonic distress vocalization (USV's, a high frequency sound emitted by rat pups in distress and thus a form of nonspecific attachment behavior) are also somewhat contradictory. Winslow and Insel (1993) report a reduction of USV's by vasopressin that was blocked by a V1a receptor antagonist, while Ijima and Chaki (2005) and Hodgson et al . (2007) report a reduction of USV's by a V1b antagonist. However, interpretation of results of USV's is difficult because the attachment system and stress systems interact heavily in this behavior. Further research is necessary to elucidate the precise role of vasopressin in infant-mother attachment. A recent article by Goodson (2008) suggests that "relative weighting of hypothalamic and medial bed nucleus of the stria terminalis nonapeptide circuitries may therefore be an important determinant of approach-avoidance behaviour". In this view, the hypothalamic vasopressinergic circuit is responsible for the stress response and the bed nucleus circuit for the affiliative behavior. Localized intracerebral administration of vasopressin or lesion studies within our experimental paradigm could be an interesting way to test this hypothesis.

In conclusion, we suggest that in the interpretation of discrimination and preference phenomena in juvenile rats, possibly in juvenile mammals in general, attachment theory should be considered. Sometimes it offers a more parsimonious and thus more elegant explanation than kin selection theory. And, although the role of oxytocin is hard to establish, the vasopressinergic reduction of the

preference for the home side shows that our experimental design has an additive value in the behavioral pharmacology of attachment.

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CHAPTER 5

Induction of partner preference in male rats and how to make it disappear.

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Submitted

Abstract.
To strengthen the face validity of the rat as an animal model for attachment behavior, we induced partner preference in the Norway rat. Male rats were housed with female rats for 2 or 24 hours, after which they were separated for 24 hours. After the separation period, males were allowed to choose between four females, one of which was the cohabitation partner. Wire mesh prevented actual reunion and the time spent in the partner zone was measured, as was the latency to the first entrance of the partner zone. In three out of four experiments, partner preference was present. In the fourth experiment, when care was taken to prevent presence of stimuli from the male subject itself, there was no partner preference. We discuss the implications of these experiments for other paradigms that involve repeated contact between animals.

Key words: Norway rat, partner preference, pair bonding, attachment theory, social memory.

Partner preference

According to attachment theory (Bowlby, 1984), a genetically programmed attachment behavioral system predisposes a young animal to develop a preference for a specific care-giver and shows attachment behavior towards this care-giver when in distress of any kind. The adaptive value lies in both nutritional and protective advantages. In humans, this attachment behavioral system continues to function into adulthood, leading to an emotional and sexual bond with a specific partner, i.e. partner preference. In rodent literature, the term 'partner preference' is used to describe the behavior in different paradigms. In rats, the term 'partner preference' has been used with regard to experiments, in which a male adult rat can choose between an unknown female rat in oestrus on the one side and an unknown female rat not in oestrus (Merkx, 1984) or an unknown male rat on the other side (Brand et al., 1991). When female sexual motivation was measured, the female could usually choose between a sexually active adult male and a castrated male (Pfeifle & Edwards, 1983).
Different operationalizations of the term partner preference have subsequently emerged. It is used in experiments where rats were able to copulate with partners that had been scented with specific odors and were later offered a choice between a scented and a natural odor partner. In males this has been termed the Conditioned Ejaculatory Preference (see Pfaus et al., 2001 for a review). The term partner preference is also used in experiments investigating the phenomenon of 'paced mating'. In these experiments, female rats are able to control the amount of sexual interaction because the male is tethered or is confined to a compartment, which has an opening that allows the female to enter and leave, but not the larger male (Avitsur & Yirmiya 1999, Paredes & Vazquez 1999, but see Meerts & Clark 2007 for a negative result). Female rats develop a preference for a partner scented with an odor that was also present during a previous experience with paced mating (Coria-Avila et al., 2005).
In voles, the term partner preference refers to the preference for a specific individual of the other sex, with which there has been prior sexual contact (see Aragona & Wang 2004, Lim & Young 2006 for reviews).The prairie vole shows this partner preference, in contrast to the montane vole, that is promiscuous (Shapiro & Dewsberry, 1990). The preference is measured in a choice between an unknown vole and a vole, with which there was a period of cohabitation, followed by 24 hours of separation. Female prairie voles showed this preference after 24 hours of cohabitation, with or without mating, after 6 hours of cohabitation with mating or after 6 hours of cohabitation without

mating, but with continuous i.c.v. infusion of 100ng/hr of oxytocin (Williams et al. 1992a). Male prairie voles showed this preference after 24 hours of cohabitation and mating, a preference that could be blocked by injection of a vasopressin V1a receptor antagonist just prior to cohabitation (Winslow et al. 1993).
In this article we use the term partner preference in the way it is used in voles. Partner preference is one of the forms of behavior, which together constitute the social organizational style of monogamy. Other aspects are paternal behavior and defense of the nest and the mate against intruders.
Because of the greater knowledge of the physiology and anatomy of rats and the problems in doing research in infant voles (the number of infants per nest is to small to use the split litter techniques that are necessary to get round between-litter variance), we are interested in the rat as an animal model of attachment. If experimental evidence of pair bonding can be found in the adult rat, this would strengthen the use of the rat as an animal model of attachment.
The social organization of rats is described in greatest detail by S.A. Barnett (1975). Rats live in groups, both in the wild and in the laboratory, if circumstances so allow. Only parturient female rats isolate themselves from the group. Barnett does not discuss the issue of monogamy vs. polygamy, but in other literature the rat is considered to be polygamous. In the research on sexual behavior carried out mainly in the 1960s collateral evidence was found for the polygamous nature of the rat. Rats that had been mating to a point of sexual exhaustion were resuming mating activity if they were presented with new partners (e.g. Fisher 1958, Fowler 1961, Hsiao 1965, Zucker & Wade 1968, Bermant et al., 1968, Carr et al., 1970, Krames 1971). This has been termed the Coolidge effect (Wilson et al., 1963). This effect is not due to a change in environmental stimulus characteristics as such, but is specific to the change in the stimulus characteristics of the female (Beach & Ransom 1967).
The protocol in our experiments was partly inspired by that used in voles (Williams et al. 1992b, Winslow et al., 1993). We used the period of 24 hours of cohabitation followed by the 24 hour interval. We did not use the three-compartment cage with one empty compartment, one for the cohabitation partner and one for a strange animal. Instead, we used a plus maze with four stimulus females behind wire mesh. The choice between four possible partners was derived from previous experiments with rat pups (Sigling et al., in preparation). In these experiments, a 4-way choice yielded a greater

effect size than a 2-way choice. In addition, we did not want any physical contact between the animals, to reduce the confounding effect of solicitous possible influence of the stimulus animals on the subject. This is why we did not allow the subjects entrance to compartments in which the stimulus animals were tethered, in contrast to the vole-experiments.

GENERAL METHODS

Animals

All animals that participated in the experiments were outbred U:WU(CPB) Albino Wistar rats and were purchased from the Animal Facility of the University of Utrecht (GDL). They arrived at the laboratory shortly after weaning and were kept in standard laboratory cages under reversed day-night conditions with lights on at 7 p.m.and lights off at 7 a.m . Testing took place in the dark period. Water bottles and RHM 11/10 chow, produced by Hope Farms Inc. of Woerden, The Netherlands, allowed for ad lib water and food consumption. Humidity was kept at ± 60% and temperature at ± 21.5 C. At the beginning of the experiments, all male rats were sexually naive and were used only once. They were between 70 and 100 days of age at the time of testing. All female rats were sterilized at least two weeks before the start of the experiments and were used repeatedly, with at least a two- week interval between experiments. Sterilization was done by ovarectomy through bilateral lumbar incisions under combined Hypnorm (0.08 ml/100g i.m.) and Dormicum (0.05 ml/100g i.p.) anesthesia. From arrival until the start of the experiments, male rats were housed 3 per cage. Female rats were housed in pairs from sterilization until the start of the experiments.

Materials

Testing took place in plus maze. The plus maze was made of acrylic glass and consisted of five sections: a central test area and four peripheral sections, each containing a female. The peripheral sections were separated from the test arena by semi-circular partitions of metal mesh. The cage was 123cm long, 25cm wide and 30cm high. A 3cm-layer of soiled sawdust, coming from the home cage of the stimulus female in it, covered the floor of each peripheral section. The floor of the central area consisted of grey plastic. Lighting was provided by a 36- Watt fluorescent red ceiling light. A VHS camcorder, connected to

a computer, recorded the movements of the male rat in the central section from above.

Methods

On day 1 of the experiment, females were placed in separate clean cages and were injected subcutaneously with 10µg of estradiol dissolved in 0.1ml of oil. On day 3, females were transferred to the experimental room and injected subcutaneously with 250µg of progesterone, dissolved in 0.1ml of oil. Four hours after injection, one male was placed in the cage of each female and they were observed for sexual contact for half an hour. The occurrence of intromission was registered and pairs in which an intromission did not occur were excluded. Observation continued until 16 pairs had reached criterion. After cohabitation, males were isolated in cages with fresh sawdust. They were placed in a different room to that of the females. the actual testing took place 24 hours after the end of the cohabitation period. Two hours prior to testing, males were placed in the central area of the plus maze for 5 minutes, to accommodate them to the testing environment. Prior to the beginning of testing, four females were placed in the arms of the plus maze, together with the soiled sawdust of their respective individual cages. After that, the four males that had cohabitated with these females were each tested successively for 15 minutes. After each male, the floor of the test area was wiped clean with a paper towel. After testing the four males with each of their cohabitation partners as stimulus animals, the females were replaced by four new females. The males with which these four had cohabitated were then tested successively and so on. The central test arena was divided in 5 zones: a central zone and the 4 zones adjacent to the cages of the 4 females. At the start of registration, males were placed in the central zone, always facing the same direction. Proceedings were recorded by a camera and scored by Ethovision (Noldus Information Technology Inc., Wageningen, The Netherlands).

EXPERIMENT 1: PARTNER PREFERENCE AFTER 24 HOURS OF COHABITATION

Method

The time of cohabitation was 24 hours. Cohabitation took place on day 3 of the experiment, separation was on day 4 and testing was performed on day 5. Females remained in their own cage where cohabitation had taken place.

Statistics

The 15 minutes of observation were subdivided into three intervals of 5 minutes each. An ANOVA for repeated measures was carried out, with interval as within subject variable. The time spent in the zones of the three non-partners was added and divided by three. Partner/non-partner zone was the between-subject variable. Latency up to the first entrance of the zone by the partner was compared with the mean of the latencies of first entrance of the three other zones. This was done with an independent t-test.

Results

Male rats spent more time in the zone adjacent to the cage of the cohabitation partner than in the zones adjacent to the other females: the between-subject factor of the partner/non-partner zone was significant ($F=6.731$, $p=.015$ in ANOVAR). The within-subject factor interval was not significant, the interaction between the partner/non-partner factor and the interval showed a trend: $F=2.834$, $p=.075$. See figure 1 for the time spent in the partner zone across the different intervals.

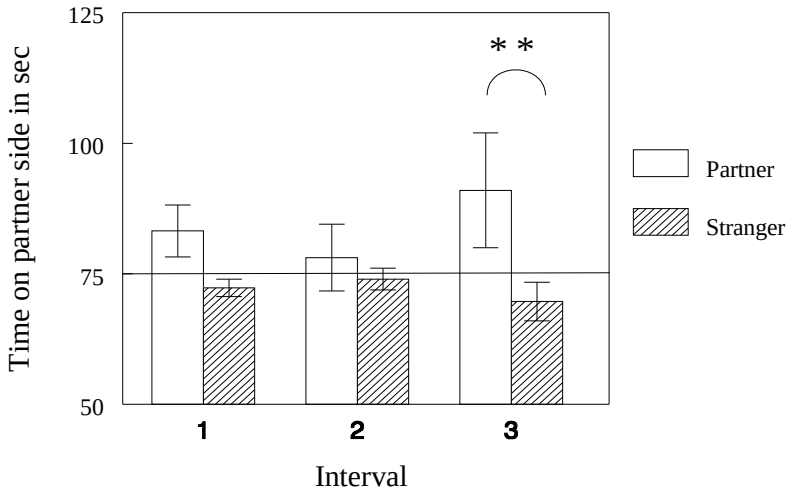

Figure 1. The effect on preference in a 4-way choice in male rats after 24 hours of cohabitation with a female followed by 24 hours of separation. The time spent in the zones of non-familiar females was totaled and divided by three. Time is sub-divided into three intervals of 5 minutes each. Error bars represent SEMs. * *: $p < 0.01$.

In a post-hoc t-test, the difference between the time spent in the partner zone and the non-partner zone in the third interval was significant: t=2.868, p=.009. The t-test on the latency to entrance of the two zone types showed a significantly earlier entrance of the partner zone: t= -2.973, p=.006, see figure 2.

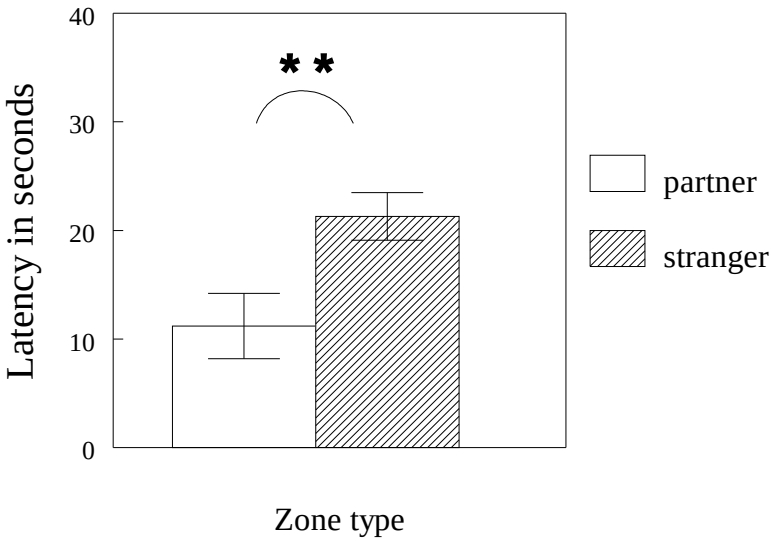

Figure 2. The effect of 24 hours of cohabitation followed by 24 hours of separation on the latency to first entrance of different zone types. The latency to first entrance of the strange females was totaled and divided by three. Error bars represent SEMs. * *: $p < 0.01$.

EXPERIMENT 2: EXTENDING THE TEST DURATION TO 20 MINUTES

Method

Because of the significance of the difference between the partner and the non-partner zone in the third and last interval in the previous experiment, the duration of observation was extended to 20 minutes in experiment 2. Otherwise, the procedure was the same as in experiment 1, as was the statistical analysis. On the basis of the previous

experiment, one-tailed testing was performed, assuming preference for the partner-zone.

Results

In the ANOVA for repeated measures, males appeared not to spend more time in the partner zone, nor were there differences across intervals, since both between-subject factor partner/non-partner and within-subject interval were not significant. The interaction between both factors was significant, F=3.959, p=.023 with Huynh-Feldt correction for violation of sphericity assumption. In post-hoc testing, a t-test revealed that in the third interval, the male animals spent more time in the zone of the cohabitation partner: t=2.676, p=.012. See figure 3 for the time spent in the home zone across intervals. The latency to entrance was again different for the partner and the non-partner zones: t= -3.139, p=.004, see figure 4.

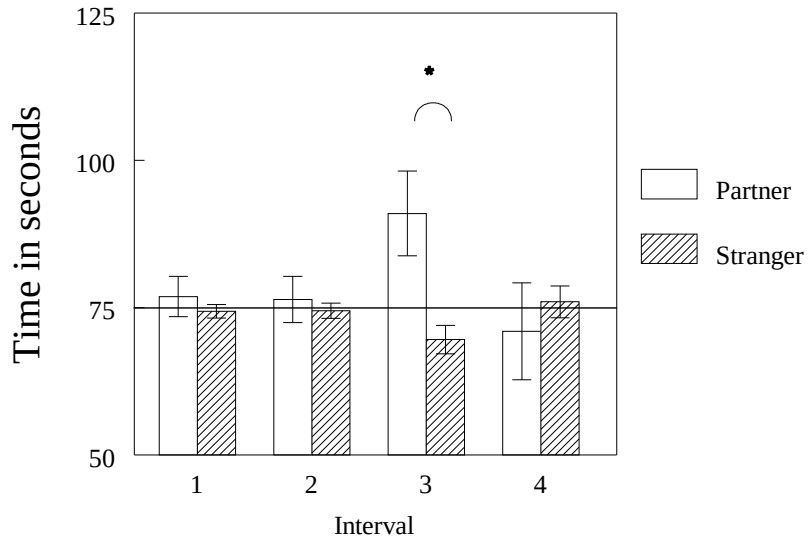

Figure 3. The effect on preference in a 4-way choice in male rats after 24 hours of cohabitation with a female followed by 24 hours of separation. The time spent in the zones of non-familiar females was totaled and divided by three. Time is sub-divided into four intervals of 5 minutes each. Horizontal line shows value expected by chance. Error bars represent SEMs. *: $p < 0.05$.

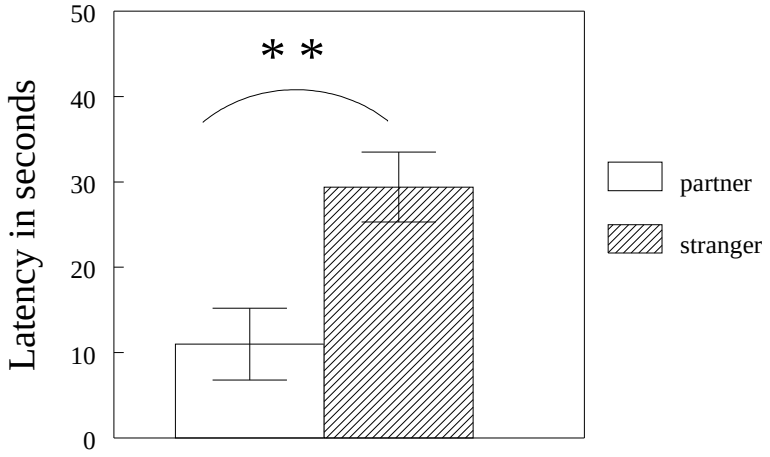

Figure 4. The effect of 24 hours of cohabitation followed by 24 hours of separation on the latency to first entrance of different zone types. The latency to first entrance of the strange females was totaled and divided by three. Error bars represent SEMs. * *: $p < 0.01$.

EXPERIMENT 3: PARTNER PREFERENCE AFTER 2 HOURS OF COHABITATION

Methods

This experiment was conducted to assess the minimum time of cohabitation needed to induce a partner preference. To this end, the cohabitation time was arbitrarily set at 2 hours. This took place on day 3 of the experiment, while testing took place on day 4, exactly 24 hours after separation of the paired animals. The time of observation was reduced from the 20 minutes of the second experiment back to the 15 minutes of the first experiment, as the fourth interval had not yielded extra discriminatory information.

Results

There were no differences across intervals, as this within-subject factor was not significant in the ANOVA for repeated measurements. Again, males spent more time in the zone of the partner, as the

between-subject factor partner/non-partner was significant: F=5.952, p=.021. There was no significant interaction. The difference in the third interval showed a trend: t= 1.842, p=.082. See figure 5 for time spent in the two zone types across intervals. The difference in latency to the two zone types was not significant.

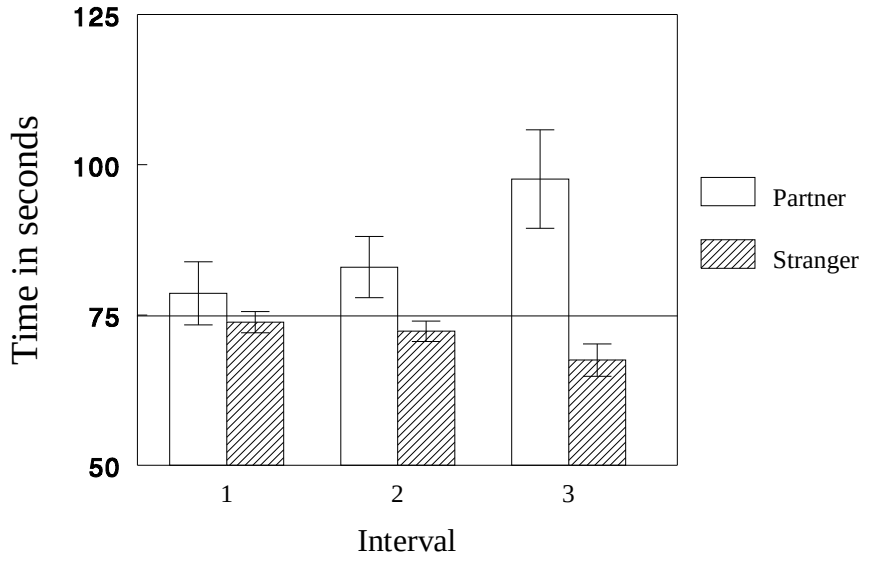

Figure 5. The effect on preference in a 4-way choice in male rats after two hours of cohabitation with a female followed by 24 hours of separation. The time spent in the zones of the strange females was totaled and divided by three. Time is sub-divided into three intervals of 5 minutes each. Horizontal line shows value expected by chance. Error bars represent SEMs.

EXPERIMENT 4: REMOVAL OF MALE OLFACTORIAL CUES

Methods

In this experiment, care was taken to remove possible male olfactorial cues from the female and the sawdust. To this end, prior to introduction of the male into the female cage, half of the sawdust from her cage was kept aside, and fresh sawdust was added to her cage. After 24 hours of cohabitation, the female was first washed with

shampoo and afterwards placed in the cage that contained the half of the sawdust that had been kept aside. This was then supplemented with fresh sawdust. Cohabitation time was 24 hours, observation time was 15 minutes and 16 pairs were used.

Results
There were no significant differences in time spent in the different zones or across intervals, as neither the between-subject factor partner/non-partner, nor the within-subject factor interval was significant. Nor was the interaction between these two factors significant. The latency to first entrance of the two zone types was not significantly different either.

DISCUSSION

In the first three experiments, there was a clear preference for the zone containing the cohabitation partner. This preference manifested itself predominantly in the third interval. The latency to entrance of the partner zone was shorter than the latency to entrance of the other zones in the first two experiments, although there was no significant preference for the partner zone in the first interval. This implies that the male rats first distinguish between familiar and unfamiliar olfactory stimuli and then quickly go on to explore the rest of the test arena. This takes about 10 minutes and in the third interval, the time spent in the familiar zone increases. In the second experiment, in which the observation time was 20 minutes, this increased preference in the third interval subsided in the fourth. This may be due to habituation to the entire olfactory stimulus complex in the test situation or it may be the result of the fact that the choice made in the third interval is not rewarded and subsequently given up.
The absence of a significant preference for the partner zone in the fourth experiment, where care was taken to remove olfactory remnants of the male rats, calls for reflection on the nature of the difference in the first three experiments.
First, the role of *sexual arousal* in the partner preference and its disappearance in our experiments must be mentioned. Warner (1927, cited in Beach & Jordan 1956) had found that if rats had copulated for two hours, “By 24 hours, the tendency of the males to cross the grid to the female had reached its high point”. Although not referring to a

choice experiment, this confers with our findings. Beach & Jordan (1956) and Bermant et al. (1968), however, showed that only a limited percentage of rats that had copulated to exhaustion were interested in re-engaging in sexual activity with new females after 24 hours of rest. In agreement with the Coolidge effect (Wilson et al. 1963), íf any sexual arousal was involved, it would have most likely been directed towards the strange females instead of the female, with which the male had cohabitated (see introduction for references). Even in the apparently unlikely case that a tendency in the males towards sexual interest in the familiar female was present, this would not have been stimulated by the properties of the female rats. They were presumably no longer receptive when testing was performed 26-48 hours after the progesterone injection.

A second explanation for our findings could have been the *aversion to stimuli of unfamiliar males.* If so, there was no positive choice for the cohabitation partner but a tendency to move away from the olfactory material deposited by the other males in the other cages, because of fear of potential conflicts (e.g. over dominance). However, aversion to stimuli from unfamiliar males is not in agreement with the findings of Latané et al. (1971) and Barefoot et al. (1975). They found no difference and a preference for novel male rats respectively, when measuring the distance between male rats of different novelty status in an open field. Aversion to strange male stimuli is also hard to explain in the light of the group living of rats in nature.
The third and most plausible explanation for the loss of preference, however, seems to be the *absence of the stimuli of the subject itself* in the sawdust. This means that in experiments 1 to 3, the male rat recognized his own soiling and, after exploration of the surroundings, turned to the most familiar olfactory environment. Griffo (1961) used the term “psychological attachment” in describing motivation for homing behavior in the cotton mouse (*Peromyscus gossypinus).* Our results suggest the fascinating possibility that pair bonding is a further developed (“higher”) form of territorial behavior and thus may make use of the same neurobiological substrate.
The findings may have implications for the interpretation of the results from other preference tests. From the description of the partner preference tests used in voles (Williams et al., 1992b), it is not clear if all precautions are taken to remove the stimuli of the experimental subject from the partner. If self-recognition is the main mechanism, differences in reactions to pharmacological interventions or between

species may be due to differences in inner states like arousal and/or need for safe territory and not to differences in pair bonding. Injection of CRF into the nucleus accumbens, for example, may induce somatic distress and thus a greater tendency to seek familiar olfactory surroundings (cf. Lim et al. 2007). Other tests in which the remnants of the subject may be an issue are the social recognition and the social discrimination test (Engelmann et al., 1995; see reviews by Popik & van Ree, 1998 and Bielsky & Young, 2004). In these tests, adult male rats are presented with a young rat and after an interval, they are presented with the same young rat (social recognition test) or with the same and a novel young rat (social discrimination test). The reduction in the time spent investigating the familiar young rat is taken as evidence of social memory. However, if the adult male rat has deposited his own olfactory stimuli on the familiar young rat during the first encounter, the test may only measure the amount that is still present on the 'familiar' young rat and not social memory.
With regard to the rat as an animal model for research into attachment, the experimental evidence presented here does not conflict with the less specific experimental evidence and more naturalistic observations in the literature on the subject of pair bonding in rats. However, our study and research on paced mating by Kippin & Pfaus (2001) and Coria-Avila et al. (2005) show the rather easy development of at least an experimental form of pair bonding that is comparable with the pair bonding as operated in prairie vole experiments. In that sense, these adult results do not discourage the use of the rat as an animal model for research into the biological substrate of attachment.

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CHAPTER 6

Effects of repeated cross-fostering on adult rat behavior.

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Submitted

Chapter 6

Abstract

Attachment theory argues that shortcomings in attachment relationships during development lead to adult psychopathology. In this context the results of two experiments are presented, in which rats were cross-fostered once or twice daily during the first three post-natal weeks. The effects of the procedure were studied on later behavior: play-behavior, social behavior, open field, elevated plus-maze, the shock-prod-bury tests and sexual behavior. Cross-fostered rats spent less time on open parts of the elevated plus-maze, showed more passive behavior in the shock-prod-bury test and male rats showed a greater latency before engaging in sexual activity. In general, female rats were less emotional than male rats. These results are in agreement with the predicted consequences of disturbed attachment early in life and with criteria for the rat as an animal model of attachment.

Keywords: Norway rat /attachment theory /separation /cross-fostering /play behavior /open field/social behavior/elevated plus-maze /shock-prod bury/sexual behavior

John Bowlby's attachment theory (Bowlby, 1984) assumes that there is a genetically programmed attachment behavioral system that predisposes infants to develop a preference for a specific care-giver and show attachment behavior towards this care-giver when in distress of any kind. Although the theory has a strong evolutionary basis, a neurobiological substrate for the behavior has not been disentangled. . We are interested in whether the rat can serve as an animal model for research into this substrate and into the consequences of disturbed attachment. Disturbed attachment has been found as a contributory factor in many studies of human psychopathology (see Sigling et al., this thesis, for a review). There are several reasons for choosing the rat as a potential animal model. First, there is a genetic homology between rats and humans. Secondly, rats live in groups, their pups are altricial during the first period after birth and the pups show a preference for their home environment (Carr et al. 1979, Sigling et al. 1998 and Sigling et al. submitted). Rats are thus comparable to humans in these aspects. Third, rat litter size allows for within-litter comparisons that are preferable in pharmacological experiments. Fourth, there is a vast amount of behavioral, physiological and anatomical knowledge about the rat that could be helpful in elucidating the neurobiological substrate of attachment. Finally, ethical and practical considerations increasingly preclude extensive neuroanatomical and pharmacological research in primates.

The vole is the animal model used most to study adult monogamous relationships (see Aragona et al. 2004 for a review). The difference between the monogamous prairie vole and the polygamous montane voles is an important reason for the interest in this animal. However, litter size in voles makes within-litter comparisons impossible. This is why, together with the reasons mentioned earlier, we have chosen the rat for studies of infant-mother attachment.

We use criteria (Sigling et al. 1998) for animal models of attachment that were modified from those suggested by Gunnar (1978): 1: preference for the putative attachment object and/or stimuli that have become associated with this object through reinforcement of attachment behavior; 2: response to the removal of the attachment object, which is distinct from the response to reduction of social stimuli per se. In previous articles (Sigling et al. 1998 and Sigling et al. submitted), we reported on a temporary preference for the home environment that reaches its peak around Post-natal Day 17. In the

present article, we focus on the second criterion: response to the removal of the attachment object. We present the results of two experiments performed to investigate the influence on adult behavior of a frequent removal of the attachment object before weaning. The frequency of removal was aimed at actually preventing the formation of an attachment bond between the pup and its mother. In the first experiment, pups were placed in a new environment once a day, in the second it was twice a day. Other differences are mentioned in the method section. It is emphasized that the procedure focused on distress as a result of discontinuity in olfactory stimuli and not on distress as a result of infantile separation or isolation (Hofer 1996; Pryce et al. 2005) or handling (Sapolsky, 1997), because our experimental set-up was designed to prevent differences in these respects. We took care to ensure there were no differences in the way pups were handled or in the time they were isolated .

With regard to cross-fostering as such: there is not much research on its effects. Cross-fostering (once, immediately after birth) is usually employed as part of the method to investigate other independent variables, not as an independent variable in itself. In rats, one time cross-fostering has had no effect on adult open-field (Amstislavsky et al., 2001) or elevated plus maze behavior (Barros et al. 2006). In our experiments, this cross-fostering is repeated. Our hypothesis was that discontinuity in olfactory home environment cues causes excess arousal during development, leading to a reduction in social behavior and an increase in anxiety-like behavior later in life.

EXPERIMENTAL DESIGN

In the first experiment, 11 pregnant Wistar rat mothers were purchased from the Animal Facility (GDL) of the University of Utrecht. They arrived 4 days before the expected date of delivery and were housed individually in standard cages in a reversed day-night schedule, with lights on at 8.00 p.m. and lights off at 8.00 a.m. This regime was maintained throughout the experiments. A layer of approximately 3cm of sawdust was provided for each cage, of which 250ml was replaced with clean sawdust every day.

Temperature was kept constant throughout all experiments at ± 21.5°

C and humidity at ± 60%. Standard lab chow and water were provided ad lib. Delivery took place within a 24-hour period, with a total of 125 pups born. Date of birth was named PND 0. Litters greater than 10 pups (8 out of 11) were culled to 10, others were left intact. Of each nest, half of the pups were randomly selected to be cross-fostered. This resulted in four groups: 26 female pups that were not fostered, 27 female pups that were, 28 male pups that were not fostered and 26 male pups that were. All pups were marked on their backs with a green felt-tip pen once every two days. From PND 10 upwards, they were marked on their tails. From PND 1 (in some litters PND 0) cross-fostering took place once a day. The procedure consisted of the removal of the mothers from the cage, followed by the removal of all pups. Pups were placed in groups in small cages with fresh sawdust. Half of the pups were then returned to their own cage and half of the pups were each transferred to a different cage. One pup from litter 1 was moved to litter 2, one to litter 3 and so on. The number of 11 litters was chosen because 11 is a prime number, which ensured that pups would be returned to their original nest only once during the 20 days of fostering. Care was taken, by alternating the order in which pups were put (back) into their respective cages, to ensure that separation from the dams and litter mates was equally long in both groups. All pups were rolled through the sawdust and placed in a huddle before the dam was returned. This was done to give all pups the olfactorial characteristics of the dam's nest, to reduce the possibility of differential behavior. The whole procedure took about 30 minutes.

Pups were weaned on PND 21 and received standard laboratory care after that (fresh sawdust once a week). They were housed in same-sex, same treatment groups of 5 in standard cages. With increasing age, group-size was reduced to 4 and 3 per cage.

In the second experiment, 22 pregnant females from the GDL were used. The split-litter procedure of the first experiment was given up, to avoid the mutual effect of the behavior of the pups of the two groups on each other, leading to regression to the mean. All pups from 11 litters were therefore cross-fostered and none of the pups from the other 11. To increase the effectiveness of the procedure, cross-fostering was now done twice a day, except on PND 1 and PND 17 when it was done only once. Cross-fostering was stopped at PND17, just before the animals were to be returned to their original nest for the

4th time. Measures were taken to ensure that the time from separation to reunion with the dam was equal in both groups. Handling was identical. In the first ten days, in both groups a plastic bottle filled with water of ± 37° C was placed in the small cage used to house the pups during the separation from the dams. Pups were placed in a huddle around and on the bottle. During the first 17 days, four pups died, two fostered and two non-fostered. Remaining groups were 45 female non-fostered rats, 54 female fostered, 51 male non-fostered and 50 male fostered pups. Otherwise, the first and second experiments were identical.

In both experiments, all pups participated in 7 behavioral tests: play behavior, open field, social behavior, elevated plus-maze, shock-prod bury and sexual behavior (males only).

The two experiments were analyzed separately. Because the split-litter design of the first experiment was given up in the second experiment, we had to account for litter-effects. For this reason, the litter of origin was entered as a covariate in the statistical analyses of the open field test, the elevated plus-maze, the shock-prod bury test and the sexual behavior test of the second experiment. Rats in the other tests were tested in pairs, always stemming from different litters. All experiments were carried out with the approval of the Ethical Committee on Animal Experiments of the Faculty of Medicine of the University of Utrecht.

BEHAVIORAL TESTS

Play behavior

Play behavior (Belles & Woods, 1964), measured in post-natal week 4, was chosen because it is the first social activity outside the nest. In play behavior, juvenile rats engage in social behavior, such as sniffing at each other, grooming each other, mounting and crawling (one rat climbs on the back or over the body of the other) but also in mock fighting that can be seen as a practicing phase for adult agonistic interactions. This mock fighting exists in behavior such as chasing and boxing-like interactions, but the prototypical element is called “pinning”. In pinning behavior, one rat lies on its back, while the other is standing over it, pinning it to the ground. Materials and methods:

testing took place in a Perspex cage 49cm high and 30cm long and wide. The floor was covered with a layer of ± 2cm of sawdust. Testing was done under red light. The day before testing, animals were brought to the test chamber and were accustomed to the test cage for 5 minutes. To enhance play behavior, animals were isolated for 3.5 hours before testing (Vanderschuren et al. 1995). Animals were observed for 15 minutes in same-sex and same-treatment couples, matched for weight, from different litters. Couples from different groups were tested alternately. The behavior was scored by an observer, using a keyboard data acquisition program (Noldus Information Technology b.v., Wageningen, the Netherlands). Sawdust was exchanged after each animal.

Statistics: MANOVA was performed with sex and treatment as independent variables. Duration of pinning behavior, social exploration (sniffing at one another) , mounting/crawling, social grooming, chasing, fighting, huddling (lying against each other without further contact) and 'other behavior' were the dependent variables.

Open field

The open field test is considered a complex measure of anxiety, exploratory behavior and general activity (Archer, 1973, Nosek et al., 2008). All three of these factors could theoretically be influenced in our design. Open-field testing took place in week 6 in experiment 1 and in week 12 in experiment 2.

Materials and method: testing was carried out individually in a round black plastic open field. The open field was 130cm in diameter with a rim 35cm high. A heavy dark brown, glass object 17cm high 7cm in diameter was placed in the center of the open field to elicit exploration in the center. A camera was placed above the open field, connected to a video recorder and a computer (Noldus Information Technology, Wageningen, The Netherlands). Animals were brought to the testing room 1 day before testing. In the second experiment, half of the animals had been isolated for two days. This was due to the social behavior test that took place a day later and required isolation (see below). Animals were tested for 10 minutes in dimmed white light,

alternating sex and treatment groups. The open field was divided into three zones: outer, middle and central. Testing started by putting the rat in the open field, always in the same place and facing the same direction. Excrement was removed with a wet sponge after each animal.

Statistics: a MANOVA was carried out with treatment and sex as independent variables. In the second experiment, isolation was added as an independent variable. As dependent variables, we chose the time it took before the rat left the outer zone to explore the middle zone (latency to middle zone) and the time spent in the central zone as measures of the balance between anxiety and exploration. In addition, total distance moved was a dependent variable as a measure of motor activation.

Social behavior

A test of social behavior was performed to measure the influence of our procedure on the balance between approach and avoidance tendencies (Spruijt et al. 1992) and was carried out in week 12. In social behavior testing, two rats are placed in an open field and their interaction is recorded automatically.

Materials and methods: in the first experiment, animals were brought to the test room the day before. Testing was carried out in the same open field described above . A camera connected to a computer was placed above the field to record the behavior of the animals. Same-sex animals from different litters from the two treatment groups were tested against each other for 10 minute, alternating the sex.

In the second experiment, half of the animals had been isolated for three days, to enhance social behavior (Latané et al., 1972). Half of the back of one rat of each pair was stained black with a felt-tip pen. This allowed for individual identification, as this rat was registered by the camera and computer as being smaller against the background of the black open field (Spruijt et al., 1992). In the second experiment, staining was alternated between the socially housed and alternated animals. Same-sex couples from the same treatment group were tested and isolated against a socially housed animal. Order of testing was

alternated between groups.

Statistics: in both experiments, a MANOVA was performed with mean distance between the two animals and total traveled distance per couple as dependent variables. In the first experiment, treatment and sex were the independent variables, in the second experiment, isolated or social housing in the three days before the experiment was an extra factor.

Elevated plus maze

The elevated plus maze was used as a fear vs. exploration test (Pellow et al.,1985; Rodgers & Cole 1994, Nosek et al., 2008) and was carried out in week 12 in the first experiment and in week 15 in the second. This test records the willingness to leave a relatively safe dark area and venture out into the light.

Materials and methods: our elevated plus maze consists of a rectangular center of 30x30cm, with 4 wooden arms connected to it at an angle of 90° to each other. Two arms are open (40 x 10cm) and two are closed (40 x 10 x 22.5cm). The maze is positioned at a height of 73.5cm in an open field. A bright white light is placed 50cm above the center. Scoring was done manually. Rats were brought to the test room one day before the experiment. Testing started by placing the rat on one of the open arms, facing the center. Rats were observed for 5 minutes and sex and treatment groups were alternated. After each rat, the maze was cleaned with a wet sponge.

Statistics: again, a MANOVA was performed with treatment and sex as independent variables. Dependent variables were the time spent on the open parts of the maze and the number of crossings from one zone to another, as measures of anxiety and activity respectively.

Shock-prod bury

In the shock-prod bury test, performed in week 13 in the 1st experiment and in week 19 in the 2nd, we measured active vs. passive strategies for coping with a non-social stressor (Trait et al., 1981). In this test, an electric prod is introduced into the cage of the rat. When the rat touches it, for instance during exploratory sniffing, the rat receives an electric shock. Initially, the rat freezes and starts hypervigilantly scanning the environment. After a variable time period, the rat engages in more active behavior, like digging a hole for itself in the sawdust or burying the electric prod with sawdust.

Materials and methods: the shock-prod bury test cage measured 25x25x35cm and was made of acrylic glass. The floor was covered with a layer of ± 1.5cm of sawdust. In the wall of the narrow side was a hole ± 2 cm above the bedding material. Through this hole, a plastic prod 6.5cm long and lcm in diameter, wrapped in iron wire, was brought into the cage. lf the rat touched the wire, a 2mA shock was delivered. Rats were transferred to the test room the day before the experiment and were placed in the test cage for 5 minutes before testing so that they became accustomed to it. Testing was carried out under red light conditions, alternating the sex and treatment groups. Testing lasted 10 minutes and behavior was again scored manually. Sawdust was changed after each trial.

Statistics: again, a MANOVA was performed with treatment and sex as independent variables. Dependent variables were the frequency of scanning behavior and immobility as 'passive' forms of coping and the frequency of burying, rearing and exploration as 'active' forms of coping. In a post-hoc analysis, active and passive behavior was pooled and analyzed in a MANOVA with the same independent variables.

Sexual behavior

The sexual behavior test (Meyerson & Hoglund, 1981), carried out in week 14 and 21 respectively, was performed to measure differences in the social (e.g. approach/initiation) aspects of sexual behavior in male rats after they had been placed in a cage with a receptive female. The sexual physiology as such, once activated, follows a rather fixed

pattern.

Materials and methods: only sexually naïve male rats were used. We expected the influence of attachment problems to be related to the initiation-phase of sexual activity, because sexual behavior follows a fixed action pattern in rats once it has been 'switched on'. Animals were transferred to the experimental room the day before testing. The female stimulus animals were ovariectomized two weeks before testing. They had been injected subcutaneously 48 hours before testing with 0.1ml of 10 microgram/ml oestradiol and approximately 3½ hours before testing with 0.1ml of 250 microgram/ml of progesterone. Testing was carried out under red light in cages 40 x 40 x 50cm, with a 3cm-layer of sawdust. Rats were placed in the test cages 10 minutes before testing in order to familiarize them with it, after which a female was introduced. Rats from the two treatment groups were tested alternately. Sawdust was exchanged after each animal. Observation lasted 30 minutes and was done manually.

Statistics: treatment group was now the only independent variable. Dependent variable was the latency to the first intermission (Meyerson & Hoglund, 1981). One latency was chosen because all latencies (e.g. up to the 1st mount, or to the 1st ejaculation, or to forms of behavior in the repetition of the sexual cycle in the same test) are necessary conditions for the occurrence of the next so that they cannot be considered independent. Measures of repetition of the sexual cycle were not eligible for the same reason and because often they, too, did not occur. A Cox regression analysis was performed, which considers the time until occurrence of a phenomenon. Standard t-tests have the problem of excluding animals that do not have a first intromission or, alternatively, assigning virtual values (e.g. total test duration) to them.

GENERAL RESULTS

There were no significant differences between treatment groups with regard to weights throughout both experiments or with regard to the day of eye-opening. Mean weights never differed more than 1%.

Results

Play behavior: in both experiments, there was neither a significant difference between male and female animals, nor between cross-fostered and not cross-fostered animals, on the amount or type of play behavior. There was also no interaction between sex and treatment. As an example, the results of the second experiment are shown (Fig. 1).

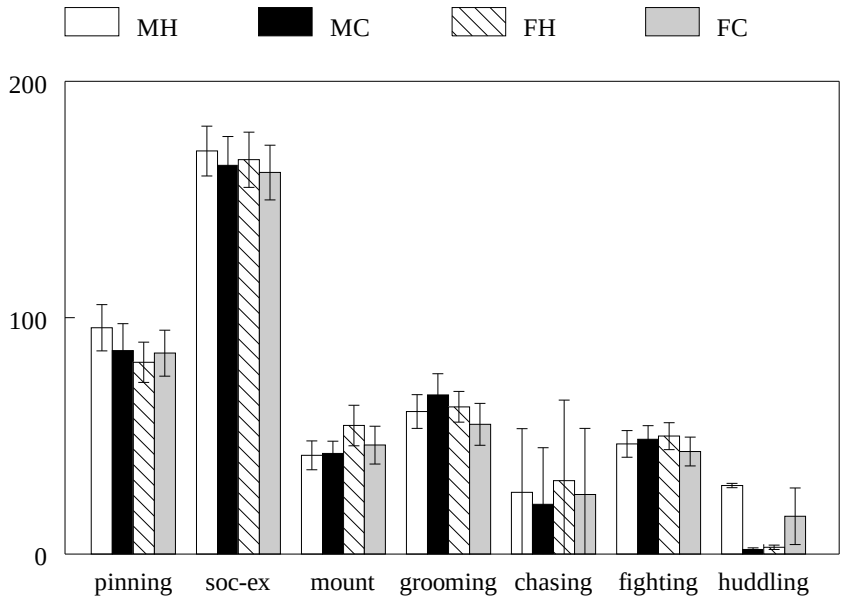

Figure 1. Duration in seconds of different forms of behavior in 15 minutes of play observation. MC=cross-fostered males, MH= non-fostered males, FC= fostered females, FH= non-fostered females. "Other behavior" category is not depicted.

Open field: in the first experiment, sex was a significant factor (n=87, F=3.01, p=.035). This was mainly due to female animals leaving the outer zone earlier (F=4.22, 13=.043 in univariate tests) and spending more time in the central zone (F=3.73, p=.057 in univariate tests, see figure 2a). Cross-fostered animals did not differ significantly from animals not cross-fostered and there was no interaction effect between sex and treatment. In the second experiment, litter of origin was not a

significant covariate: n=202, F= 0.103, p=0.958. Both sex (F=3.93, p=.009) and isolation status (F=8.40, p=.000) significantly affected results. The influence of sex was similar to the first experiment: female rats had a shorter latency to leave the outer zone (F=10.25, p=.002 in univariate tests, see fig. 2b). The effect of isolation was predominantly expressed in a reduction of the traveled distance (F= 23,77, p=.000 in univariate tests, see fig. 2c). Cross-fostering treatment was not a significant variable, nor were the different interactions.

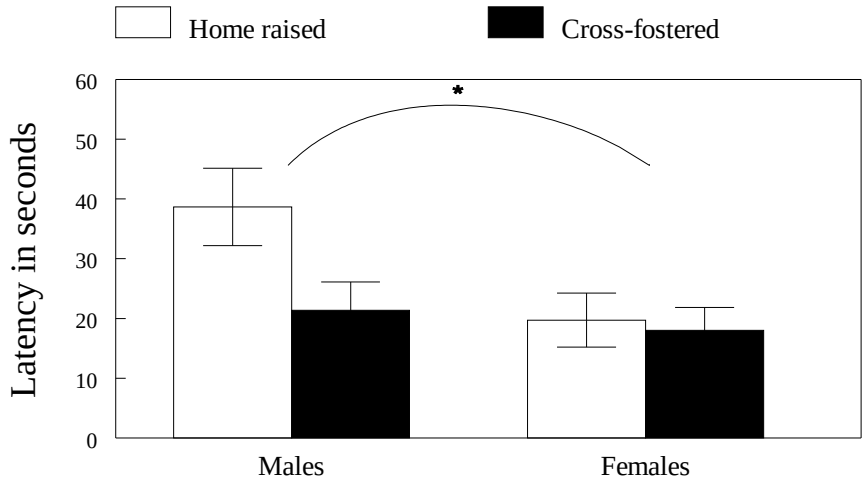

Figure 2a. Latency of leaving outer zone in open field test. Cross-fostering once daily. Sex difference was significant (F=4.22, p=.043 in univariate testing). Error bars represent SEMs.

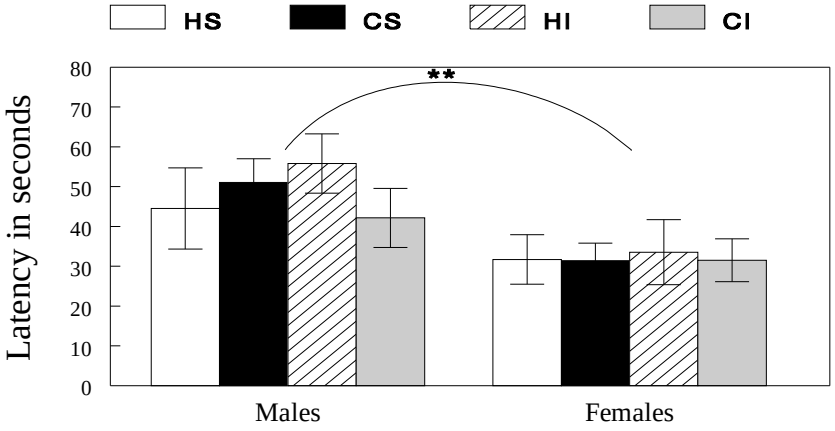

Figure 2b. Latency of leaving outer zone in open field test. Cross-fostering twice daily. Half of the animals were isolated two days prior to testing. Sex difference was significant (F=10.25, p=.002 in univariate testing). Error bars represent SEM's. HS=home raised/socially housed, CS=cross-fostered/socially housed, HI=home raised/isolated, CI=cross-fostered, isolated.

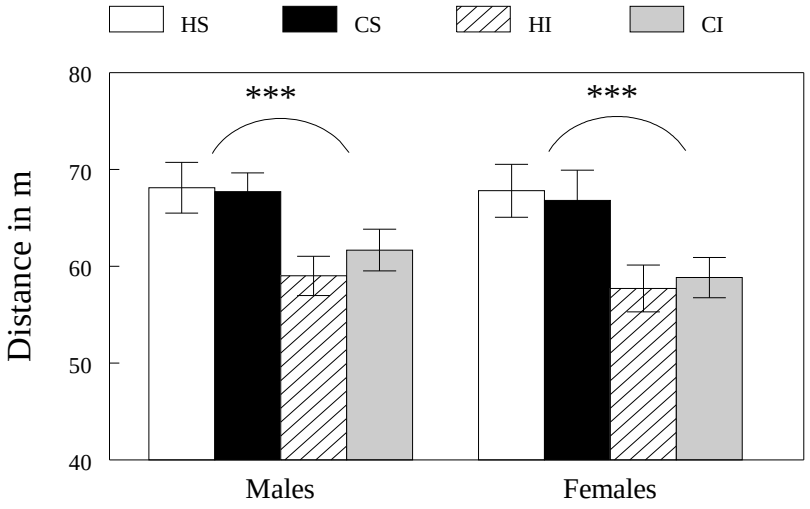

Figure 2c. Total traveled distance in open field. Cross-fostering twice daily. Half of the animals were isolated two days prior to testing. Soc = socially housed, Iso = isolated. Isolation factor was significant (***=p <.000). Error bars represent SEMs. Legends see fig. 1b.

Social behavior: in the first experiment, cross-fostering treatment and sex had neither a significant influence on the mean distance between the rats nor on the total distance moved. There was no interaction effect between treatment and sex. In the second experiment, isolation vs. social housing prior to testing was not a significant factor, so data were pooled. Sex was a significant factor (n=100 pairs, F=16.97, p=.000): the traveled distance was greater in female rats (F=16.64, p=.000 in univariate tests), see fig. 3a, as was the distance between the animals (F=16.75, p=.000 univariate), see fig. 3b.

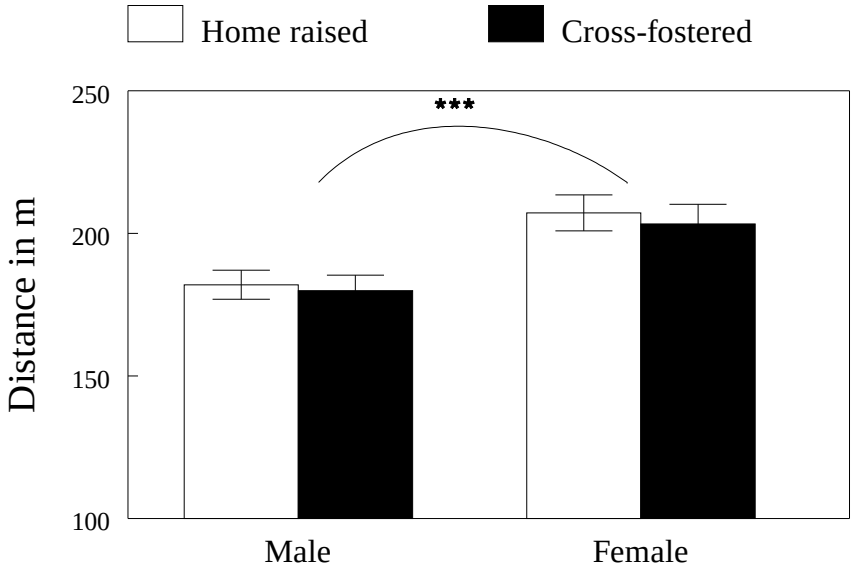

Figure 3a. Social behavior test. Influence of sex on total distance moved per couple. Cross-fostering twice daily. Data from animals housed socially and isolated prior to testing are pooled. Sex was a significant factor (***= p<.000). Error bars represent SEM's. n= 24-26 per group.

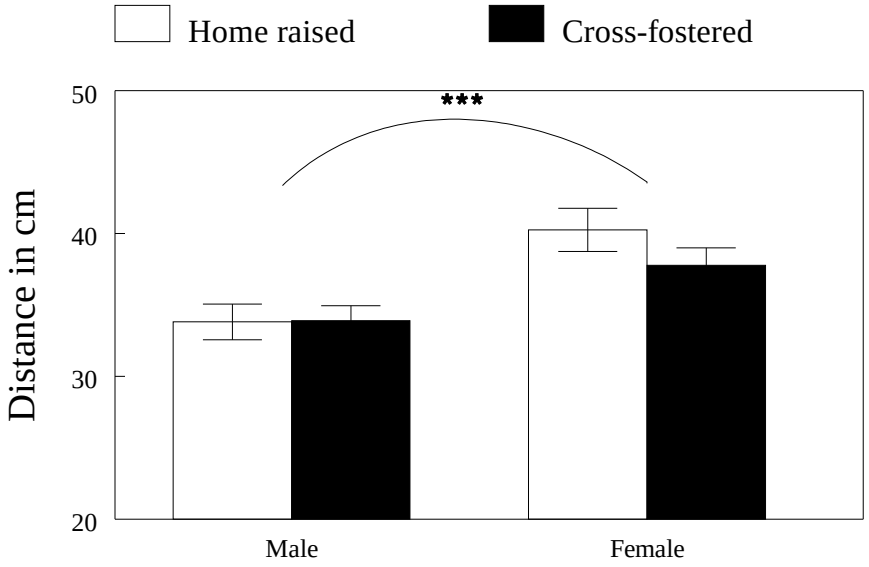

Figure 3b. Influence of sex on mean distance between rat pairs during social behavior testing. Cross-fostering twice daily. Data from animals housed socially and isolated prior to testing are pooled. Sex was a significant factor (***=p <.000). Error bars represent SEM's. n= 24-26 per group.

Elevated plus maze: in the MANOVA of the first experiment, female animals differed significantly (N=106, F=8.78, p=.000) from male animals. Females appeared to be less fearful, spending more time on the open parts (F=9.88, p=.002 in univariate testing) and crossing more often from one zone to another (univariate F=17.56, p=.000, see fig. 4a).Treatment and interaction effects were not significant. In the second experiment, litter of origin was a significant covariate: N= 201, F=5.290, p= 0.006 and was included in the analysis. Cross-fostering was a significant factor (F= 3.281, p=.04) resulting in cross-fostered animals spending less time on the open parts (see fig. 4b) and crossing less often from one zone to another. Female animals again appeared to be less fearful (F= 3.718, p=.02). There was no significant interaction.

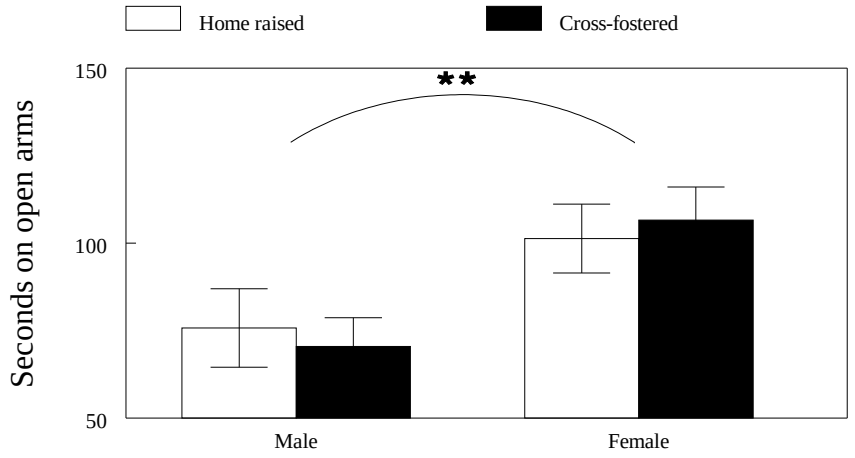

Figure 4a. Influence of sex a on time spent on open arms during elevated plus-maze testing. Cross-fostering was performed once daily. Sex was a significant factor (**=p<.01). Error bars represent SEM's. n=24-26 per group.

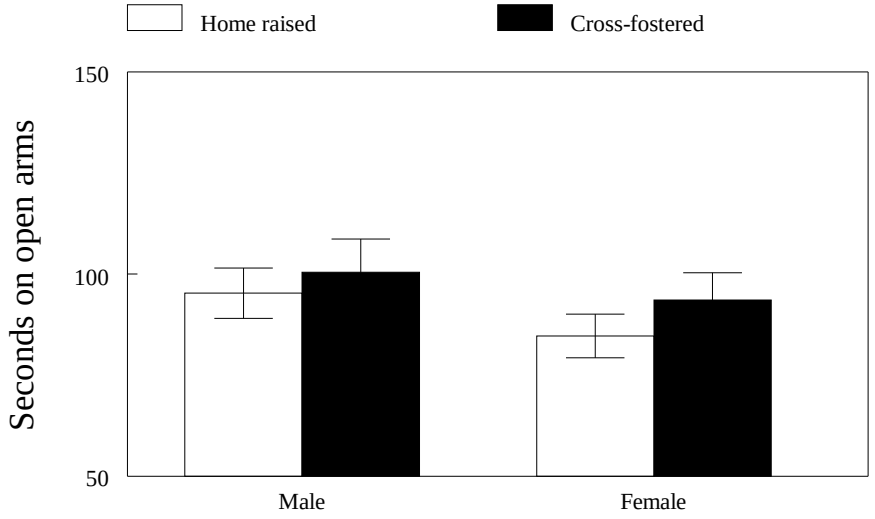

Figure 4b. Influence of sex and cross fostering status on time spent on open parts during elevated plus-maze testing. Cross-fostering twice daily. Both sex (F=3.56, p=.03) and cross-fostering status (F=4.38, p=.014) were significant. Error bars represent SEM's. n=48-52 per group.

Shock-prod bury test: the MANOVA of the behavior showed a trend in the effect of the cross-fostering treatment in experiment 1 (n=104, F=2.21, p=0.059). This was largely due to an increased frequency in scanning behavior (F=6.23, p=.014 in univariate tests). Sex was not a significant variable, and there was no interaction effect between treatment and sex. In experiment 2, sex was a significant factor (n=204, F=4.41, p=.001), with female animals showing less active coping. Cross-fostering treatment effect was not significant with regard to the individual behavior and there was no interaction between sex and treatment. The litter of origin covariate was not significant, F=.590, p=0.70. In the pooled behavior, the frequency of the passive behavior category was greater in the cross-fostered group in experiment 1 (F=5.85, p=.017), see fig. 5a. Although this was largely due to male rats, sex and interaction between sex and treatment were not significant factors. In experiment 2, the frequency of the active behavior category was decreased in the cross-fostered group (F=4.65, p=.032, see fig. 5b). The sex factor was only significant in the passive category (F=14.73, p=.000), where the frequency of the behavior was greater in females. There were no significant interactions in experiment 2.

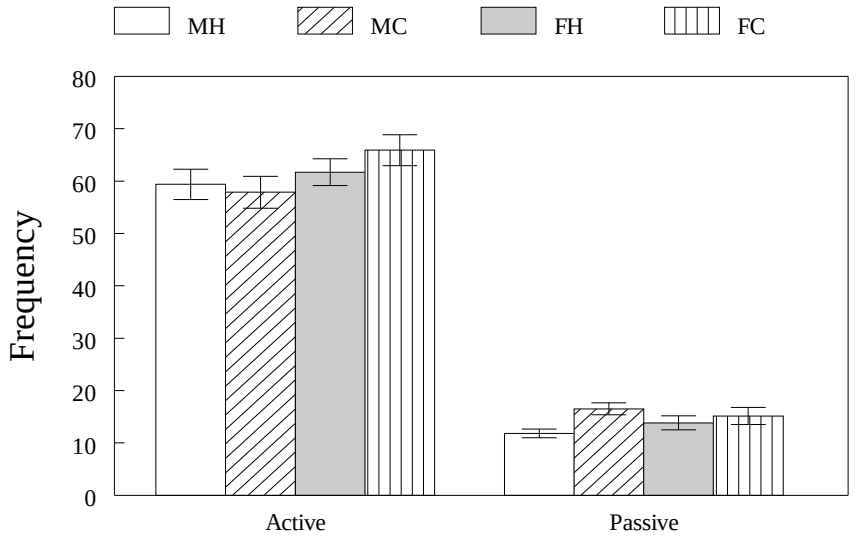

Figure 5a. Influence of sex and cross fostering on frequencies of active and passive behaviors in shock-prod bury test. Cross-fostering once daily. Fostered groups were more passive (F=5.85, p=.017). Error bars represent SEM's. MH=male/home, MC=male/cross-fostered, FH=female/home, FC=female/cross-fostered.

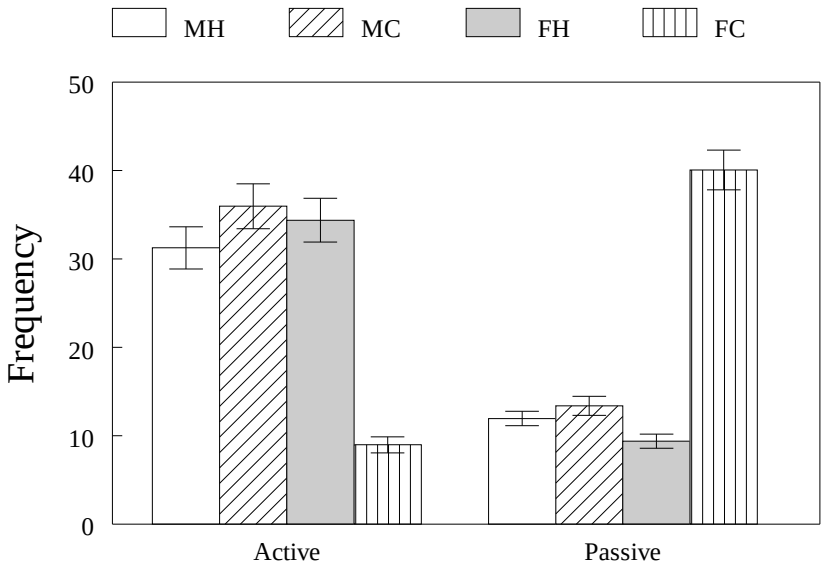

Figure 5b. Influence of sex and cross fostering on frequencies of active and passive coping behaviors in shock-prod bury test. Cross-fostering twice daily Fostered groups were less active (F=4.65, p=.032) and females were more passive (F=14.73, p=.000). Error bars represent SEM's. MH=male/home, MC=male/cross-fostered, FH=female/home, FC=female/cross-fostered.

Sexual behavior:

In the first experiment, the latency to intromission was increased by the cross-fostering procedure (n=39, Wa1d=5.33, p=.021), see fig. 6a. In the second experiment, the same effect occurred (n=90, Wald=5.97, p=.015), see fig. 6b, while the litter covariate was not significant (Wald=0.053, p=0.82). To illustrate the progression of the sexual cycle in the different groups, latency to mounting and latency to ejaculation are also depicted. The results of the non-performers are not included in these figures.

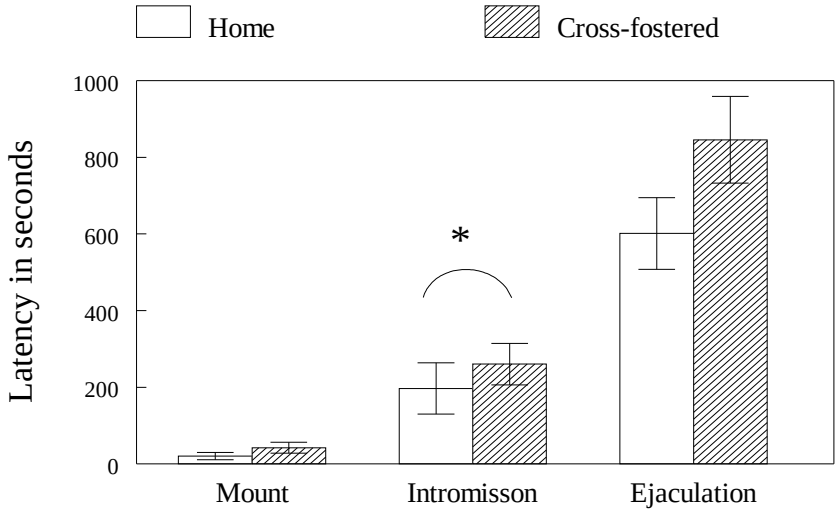

Figure 6a. Influence of cross fostering on latencies of different behaviors during sexual behavior testing in male rats. Cross-fostering once daily. Intromission was delayed (*= p <.05). Error bars represent SEM's.

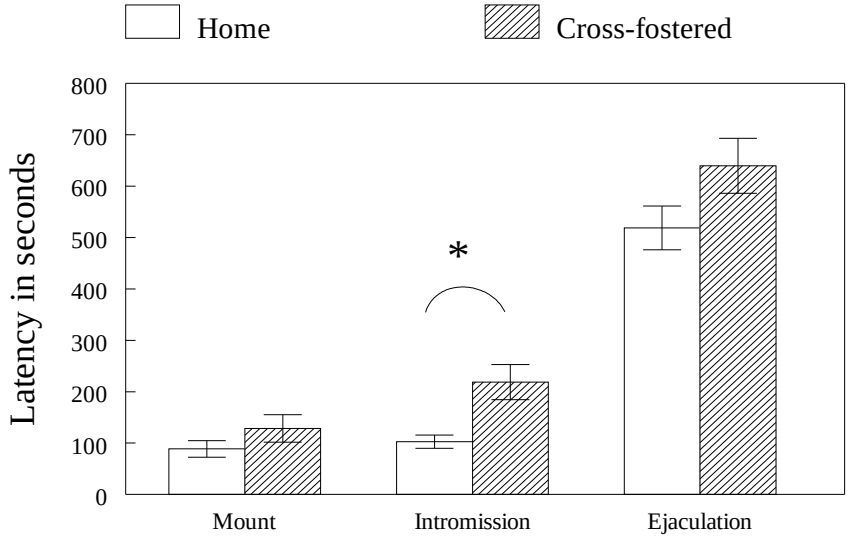

Figure 6b. Influence of cross fostering on latencies of different behaviors during sexual behavior testing in male rats. Cross-fostering twice daily. Intromission was delayed (*= p<.05). Error bars represent SEM's.

DISCUSSION

There were no differences in the *play behavior* test. Our hypothesis was that cross-fostering would lead to reduced pinning, fighting and reduced social interest in general. This was based on a report by Shimozuru et al. (2007), who compared an early weaning group (on PND 16) with a group that was weaned on PND 30. They found reduced pinning and fighting in the early weaning group. . As our intervention also consisted of a reduction of normal mother-infant contact, we expected comparable results in our cross-fostered groups, but there were no differences. The absence of differences between the sexes, independent of treatment, was in striking contrast with the literature (see Pellis, 2002, for a review). The decision to use same-treatment couples was made because dominance differences within pairs are not easy to observe experimentally (Symons 1978, Pellis & Pellis 1991) and we hoped to augment the between-group differences by using same treatment pairs. It is possible that this was the wrong choice and that using pairs from a different treatment group would have yielded results. Another explanation for the lack of results could lie in the nature of the play behavior. It may be such a robust, genetically pre-programmed behavior that a more radical intervention than ours is needed to create group differences.

In the *open field* test we did not find significant differences in the latency to leave the outer area as a measure of the balance between avoidance and exploration. We expected a shift in the direction of avoidance, as 60 minutes (Knut & Etgen, 2007) and 180 minutes (Vazquez et al., 2005) of maternal deprivation per day have this influence. Maternal deprivation for 5 hours in the presence of litter mates also induced increased anxiety (Rees et al., 2006). On the other hand, Roman et al. (2006) did not find an effect of a 6-hour separation from PND 1 until 21. Benetti et al. (2007) exposed a group of infant rats up to PND 10 to 3 minutes of handling outside the nest. This increased exploratory activity in an open field compared with 3 minutes of handling inside the nest. Brief separation and handling on PND also reduced anxiety when compared with an undisturbed control group (Madruga et al. 2006). The same was found after 15 minutes of handling on PND 2-21 (Cannizzaro et al., 2005, 2006). Our negative results may be due to the form of our open field, maybe an open field with corners (Vallée et al., 1997) being more sensitive in

this respect. The fact that the traveled distance did not differ between groups could be due to the ambivalent nature of this measure. In a principal component analysis (Vallée et al., 1997), open-field distance correlated positively to both an exploration and an escape/avoidance factor. This could, depending on the specifics of the treatment and the test situation, lead to the elimination of differences. The reduction in the traveled distance as a result of pre-test isolation of two days could be speculatively seen as increased anxiety-like behavior or as an energy conservation reaction to the isolation.

With regard to the *social behavior test*, cross-fostering did not have a significant effect. Earlier studies in our lab showed that isolation during post-natal weeks 4 and 5 decreases social behavior (van den Berg et al., 1999), an effect we also expected from our cross-fostering procedure. Maciaq et al. (2002) had also found reduced social interest following 5 8-hour separation sessions every other day from PND 2 up to PND 10. The lack of significant results in the present study may be the consequence of the use of the automatic tracking system. The tracking system (Spruijt et al., 1992) is excellent at scoring distances and directions of movement, but has difficulty in distinguishing between various types of behavior. The fact that the added factor of isolation in the second experiment did not increase social behavior is in contrast with previous findings (Latané et al., 1972, Spruijt et al. 1992).

The *elevated plus maze* showed the first significant difference between cross-fostered and non-cross-fostered groups. In the second experiment, cross-fostered animals spent less time on the open arms. The results were in accordance with our expectations and, as a number of authors have demonstrated, increased anxiety-like behavior as a result of early deprivation. More recent reports are from Kalinichev et al. (2002), Daniels et al. (2004), Park et al. (2005) and Lee et al. (2007), who found this after daily separations from the dam of 180 minutes during the first two weeks. Knuth and Egden (2007) report reduced exploration after daily 1- hour separations on PND 4-9. Others, however, did not find an effect of 3 hours (Greisen et al., 2005b, Slotten et al., 2006), 4 hours (Marmendal et al., 2004) or even 6 hours (Roman et al., 2006) of maternal separation in the first weeks. Exploration of open arms is increased as a result of 15-minute daily separations (Cannizzaro et al., 2005, 2006). The difference between the elevated plus maze result and the negative results in the open field

may be due to the fact that the elevated plus maze is considered a more specific measure of anxiety (Rodgers & Cole, 1994).

There was an increase in passive coping in the first and in the second *shock-prod bury* experiment, in line with our hypothesis. One could expect two opposing mechanisms in our experiments: increased arousal and anxiety as a consequence of destabilization in the early environment ("disturbed attachment") and, from a different theoretical perspective, reduced arousal and anxiety because the repeated change of olfactorial stimuli has an effect comparable to handling ("enrichment"). In accordance with this, Arakawa (2007) found a decrease in active coping following isolation from post-natal day 26 to 40, while an increase in active coping is reported as a result of daily handling, compared with an undisturbed control group (Meerlo et al., 1999, Roy & Chapillon, 2002). Apparently, in our experimental set-up, possible enrichment-type effects are overridden by the disturbance of attachment. The lack of significant differences in the analysis of the individual behavior may be due to the fact that the behavioral categories scored in shock-prod bury experiments, although generally accepted, are not manifestations of specific functions.

In male rats we found an increased latency in engaging in *sexual behavior* in both experiments. This was in line with our expectations, based on the greater part of the literature on the subject. According to Rhees et al. (2001), six hours of isolation during PND 2 to 10 resulted in decreased sexual behavior. Benetti et al. (2007) found the same following daily handling outside the nest for 3 minutes in the first 10 post-natal days, as opposed to 3 minutes of handling inside the nest. Handling and aversive stimuli during PND 1-10 decreased sexual motivation (Padoin et al., 2001). Twenty minutes of isolation and 1 minute of handling daily from PND 1-14 reduced sperm quantity and quality on PND 61 (Mazaro and Lamano-Carvalho 2006). Greisen et al. (2005a), however, found increased sexual motivation after daily 180-minute separations on PNDs 2-14, as compared with daily separations of 15 minutes. We presume that the increased latency in engaging in sexual behavior in our experiments is an expression of a higher level of anxiety, induced by the instability of the home environment, manifest on the social level. Once sexual behavior was started, the time to completion was not influenced by the cross-fostering procedure. This is probably due to the fairly automatic procedure of the physiology of this behavior.

The sex differences were consistent. In the open field test, the social interaction test, the elevated plus maze and the shock-prod bury test females showed less emotionality than male rats, an interesting finding considering human stereotyping.

In all five tests that showed an effect of cross-fostering, the effect was comparable with the consequences of longer-lasting maternal deprivation (Pryce et al., 2005): an increase in anxiety-like behavior. Focusing purely on the physical aspects of the procedure, all results would be expected to be analogous to handling or enrichment experiments. Handling and enrichment increase neuronal outgrowth, synaptic density, etc. One can assume that this is the consequence of increasing the amount of non-noxious stimuli during development. The repeated confrontation with novel olfactory stimuli in our experiment should by analogy lead to reduced anxiety-like behavior. The fact that we found the opposite means that the quantity of stimuli does not suffice as an explanation here. We argue that it is the stressfulness of the discontinuity in the quality of the stimuli that causes the effect and we think our experiments contribute to meeting the second of the criteria for an animal model of attachment mentioned in the introduction: 'Response to removal of the attachment object which is distinct from the response to reduction of social stimuli per se'.

Apart from the direct influence of the experimental procedure on the pups, there is also the possibility that the pups induce differential care in the dams through their behavior, most likely through the ultrasonic distress vocalizations (USVs). We have tried to record USVs during the first experiment with an ultrasonic microphone within the cages before returning the dam. We have never recorded any USVs and thus did not include it in our second experiment. We believe this absence of USVs is due to placing the pups back in the cage in a huddle. In a huddle, pups lack the two main incentives for emitting USVs: cooling down and loss of tactile contact with a conspecific (see review by Hahn & Lavooy, 2005). Another reason why USVs are unlikely to explain our results stems from an experiment by Conely and Bell (1978). They showed that isolated pups confronted with nest material of their own mother produced less ultrasonic distress vocalizations than pups confronted with nest material from another lactating female.

This means that the fostered pups would elicit more maternal care

(Brouette-Lahlou et al., 1992) than the non-fostered pups and this would lead to reduced emotionality in fostered pups (Liu et al., 1997; see Macri & Wurbel, 2006, for a recent critical review), which is not what we found. But it cannot be excluded for certain that other aspects of the pups' behavior lead to differences in maternal care and to differences in adult behavior. Yet we do not believe this to be in conflict with our hypothesis that it is the stressful experience of discontinuity in the pups that leads to changes in adult behavior.

An alternative mechanism could be that the pups did not influence maternal behavior, but that dams themselves distinguished cross-fostered from non-fostered pups. This seems unlikely for a number of reasons. First, pups were rolled through the sawdust of the dam's cage before the dam was put back into it. Second, pups in the first experiment were put back into a mixed-intervention huddle after being rolled through the sawdust. Thus, any olfactory remnants from the previous environment would soon be transferred to non-fostered pups. Third, in the second experiment, dams would only encounter either non-fostered or cross-fostered pups, so they could not differentiate their behavior. Theoretically, however, the twice-daily confrontations with traces of previous environments in the cross-fostered group could have caused stress in these dams, causing differential behavior. Yet in hours of unstructured observation before, during and after the cross-fostering proceedings, we never saw any sub-group formation in the first experiment, nor did we see any differences in interaction between the two groups in the second experiment. This means that pups spent nearly 100% of their time in the huddle and dams spent 90 % of their time nursing and licking them. Moreover, although dams can in principle identify their own from strange pups (Beach & Jaynes, 1956), this is thought to be regulated mainly by recognition of their own, maternal stimuli on the pup (Beach & Jaynes, 1956, Bauer, 1993). These were provided by rolling the pups through the sawdust and placing them in a huddle, making it unlikely that dams perceived cross-fostered pups as strange.

To conclude, we argue that rat pups need continuity in the cues that identify the mother and the home environment for normal development. Absence of this continuity leads to changes in adult behavior that can generally be labeled as increased emotionality or anxiety-like behavior. We believe we have shown it to be plausible, in this and previous articles (Sigling et al., 1998, Sigling et al.,

submitted), that the rat can serve as an animal model for research into the neurobiological substrate of maternal attachment.

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Chapter 7

General discussion

The central question of this thesis is whether or not the rat is a suitable animal model for attachment. In the first chapter, the content of attachment theory is described and the impact on psychiatry with a review of the influence of disturbed attachment on psychopathology. In the second chapter, rodent studies with relevance to attachment theory are discussed. In the third chapter a study on the course of a preference for the home cage during the pre-weaning stage of rat development is presented in a two-way model of choice. In the fourth chapter, this course of preference is confirmed in a four-way model, a stronger preference in females is described and it is shown that only familiarity, and not genetic relatedness influences the preference. In the fifth chapter, the induction of a partner preference in adult male rats is shown and its disappearance, after careful removal of possible remnants of this male before testing. The sixth chapter describes the consequences in adult behavior of the prevention of attachment relations during infancy. It is demonstrated that this leads to an increase in anxiety-like behavior and a decrease in sexual behavior. This last chapter discusses a number of issues that are presented in the thesis and tries to integrate animal and human evidence where necessary.

The pathway from attachment to psychopathology

In chapter 1, the relationship between attachment and psychopathology in humans is reviewed. The evidence is overwhelming that disturbances in attachment lead to psychopathology. This is the result of the fact that, apart from simple survival, the attachment relationship the infant has with its mother has great developmental consequences. In a healthy attachment relationship, important psychosocial skills are learned through interaction and imitation. Language is the most conspicuous of these, but regulation of affect, and thereby the calibration of the stress systems, in particular the HPA-axis, are also very important. Actual experiences of adequate alleviation of distress by the care-giver create

the mental image in the infant of the outside world, i.e. the care-giver being available reliable and efficient. The behavior of care-givers contingent on attachment behavior (behavior that is directed at promoting proximity of the attachment figure, resulting in food or shelter or other types of care) create the mental image in the infant of the self being capable of organizing support when necessary. The reduction in stressful internal states such as hunger, pain or sleep within a reasonable time span through the intervention of the care-giver organize the biological homeostatic mechanisms within the infant in an effective way. But affective states and intensity of emotions are also regulated in these interactions. Positive affective exchanges through babbling and talking help in developing language, which is subsequently instrumental for the child in influencing the (social) environment. Therefore a positive image of significant others and the world, a positive image of the self as being efficient in organizing support, homeostatic mechanisms in stress regulation that function well, affects and emotions that appear when necessary and are not excessive in intensity or duration, and an adequate mastery of language all are the consequences of a secure attachment relationship during development. It can be inferred from this list of positive consequences that (major) shortcomings in the attachment relationship can have negative consequences for well-being and lead to psychopathology, as illustrated in Chapter 1.

Reasons for the lack of acceptance of attachment theory in clinical practice

However, although there are many ways, as illustrated above, in which shortcomings in attachment relationships can lead to negative consequences, the classification of these shortcomings is quite simple. In most instruments or methods of classification there are only three insecure types of attachment: anxious (preoccupation with the relationship to the attachment figure), avoidant (a detached attitude towards attachment), or disorganized (no clear compensatory strategy towards the attachment figure). A lot of research has focused on correlating these classifications to psychiatric disorders according to the DSM classification system. This is entirely understandable in a psychiatric culture dominated by a categorical, and not a dimensional, diagnostic system. However, this has led to an impressive, but rather repetitious, literature in which all kinds of psychiatric disorders are correlated to just a few attachment categories, often times only two (secure vs. insecure). This has evidently led to little enthusiasm in

clinicians. There is a paucity of research that aspires to connect attachment disturbances to psychopathological phenomena in a more meaningful, causal way. For instance, the literature concerning negative childhood experiences and disturbances of normal HPA-axis functioning is largely written from the perspective of the impact of traumatic events such as maltreatment, abuse or parental loss. There are just a few studies (e.g. Rifkin-Graboi 2008, Scheidt et al., 2000) that connect the impact of attachment history, as reflected in the Adult Attachment Interview, with the HPA-axis. In addition, we could find only one study (Salzman, 1997) on the relationship between the AAI and affective instability or impulsivity. The vast body of research reviewed in Chapter 1 on the relationship between attachment classification and psychiatric classification has demonstrated beyond doubt that disturbed attachment is an important factor in the causation of psychopathology. However, thinking along the lines of attachment theory is far from ubiquitous in adult psychiatry. Attachment researchers are partly responsible for this because they have focused too little on the integration of the biological and psychological aspects of attachment theory (Schore, 2000) and too much – maybe also to increase acceptance – on DSM- classification-oriented research. For many years now, psychoanalysis has not been accepted as a valid theory of developmental psychology. It has not been replaced by any other in clinical psychiatry. Although the situation in child and adolescent psychiatry is somewhat different, the title of the 2007 book "Attachment theory in clinical work with children: bridging the gap between research and practice" (Oppenheim & Goldsmith, Eds.) shows there is room for improvement.

The dearth of research integrating biological and psychological aspects of attachment has unfortunately coincided with the over-emphasis on neurobiology that psychiatry has experienced in recent decades. Although this has not brought a single advance with regard to diagnosis or treatment of any functional psychiatric disorder since the arrival of the atypical antipsychotic clozapine in 1975, it has clearly suppressed attention (and funding) for psychologically-oriented research. This over-emphasis on neurobiology took place in the same era that theorists, clinicians and policy-makers in psychiatry generally declared themselves adherents to the bio-psychosocial model (Engel, 1977). The term bio-psychosocial model is misleading however, because the model as such does not exist. It is only shorthand for saying that biological, psychological and social factors interact in disease and should all be attended to. Strangely enough, in this bio-

psychosocial age- the only real bio-psychosocial theory that exists lacks acceptance. Attachment theory claims relevance to the biological (a hard-wired behavioral system that has shown selective advantages during evolution), psychological (cognitive working models of the self and significant others) and social domain (resulting from and in behavior in intimate relations) and interactions between them.

A third reason for the present status of attachment theory is that there is very little evidence about therapeutic possibilities. Randomized trials are very scarce and limited to (young) children (e.g. van Zeijl et al. 2006, van Doesem et al. 2008, Dozier et al. 2008). They are usually directed at improving parenting in at-risk groups. In adults, attachment variables are sometimes used as mediators of psychotherapy (Mc Bride et al., 2006) or as an outcome measure (Levy et al., 2006). We do not know of any randomized study in children or adults with an intervention based on attachment theory aimed at a specific disorder, although the van Zeijl study was carried out on children that scored high on a measure of externalizing behavior. In adults, we have not found any randomized study at all with an attachment-based intervention or therapy. Thus, the use of attachment theory in etiology and diagnosis of disorders does not lead to the use of specific evidence-based interventions because they do not exist. This, too, is an important obstacle in the acceptance of attachment theory in clinical practice.

Attachment theory in pre-clinical research

The literature on rodent research reviewed in Chapter 2 does not to a large extent originate from an attachment perspective. There are some exceptions however, usually concerning UltraSonic distress Vocalizations (USVs, high-frequency sounds emitted by rat pups in reaction to separation or physical distress). The work of Myron Hofer and co-workers in particular should be noted. They selectively bred two lines of rats that had either high or low intensity of USV response to isolation on post-natal day 10 (Brunelli and Hofer, 2007) and assume this corresponds to different attachment strategies in humans. Other exceptions are Insel and Winslow (1991) and Nelson and Panksepp (1996), although not explicitly referring to Bowlby, working in a perspective of infant-mother attachment. In higher animals there is, of course, the work of Harry Harlow (Blum, 2002). He showed that pleasant tactile stimuli do generate more attachment behavior in young monkeys than a milk bottle. This contradicted Freud's

secondary drive theory, which stated that affection for the mother was secondary to her providing food.

Generally speaking, however, attachment theory is not mentioned in pre-clinical research. This can be partly explained by the fact that pre-clinical research has always for the most part been physiological or anatomical in nature. It builds on previously gathered data of a very specific nature without requiring theory. In behavioral research, Skinner's behaviorism paradigm led for a long time. It discouraged interpretation or theorizing about internal phenomena and focused on observable behavior. The somatic and behaviorist tradition of pre-clinical research continues to dominate unwritten rules about sticking to the data and not theorizing too much. In this type of discourse, any theory is unlikely to flourish.

Another factor is that Bowlby originally intended his writings to influence the thinking and practice of his clinical colleagues and not that of the research community. Later, his ideas were taken in by academic developmental psychologists, who did not have a history of animal research. This means that, with the above-mentioned exceptions, attachment ideas did not circulate in the departments where the animal research is done.

Thus, the review in Chapter 2 is an attempt to look at research that has mostly been done for reasons other than from an attachment point of view. This research was aimed at elucidating sensory development, nipple attachment, learning, stress response, etcetera. It is debatable whether such a re-interpretation is acceptable. It could be argued that each experiment would have been executed somewhat differently if it had originated from an attachment perspective and could then have had a different outcome. On the other hand, if a rat pup prefers his mother to an unknown female in an experiment designed to measure maturation of the olfactory system, it is unlikely that the outcome would have been different if preference for the mother was the starting point. Bowlby referred extensively to the animal literature, especially in the first part of his trilogy Attachment and Loss, and he, too, was forced to use data that were gathered for other reasons. And now, some 50 years later, there is still a significant deficiency in attachment theory-based animal research, which forces us to study literature that originated from another framework. The conclusion that can be drawn from the literature reviewed in Chapter 2 is that there is sufficient evidence to make it plausible that rats do have a behavioral system, which gives rise to a preference for a specific stimulus context and organizes signaling and other proximity-promoting behavior towards

that complex. This makes further investigation worthwhile on the possible role of the rat as an animal model for attachment.
In Chapter 3, the preference for the home side during a specific phase of development is shown in a two-way model of choice and confirmed in Chapter 4 in a four-way choice. Although this is not mentioned explicitly in Chapter 4, the experiments concerning the effects of cross-fostering and of vasopressin and oxytocin mentioned there also show a clear preference during the third week for the home side. Such a consistency is not always present in behavioral experiments in rats. However, one could say that a preference that is only manifest in the third week is not very relevant for attachment theory. This critique can be answered in three ways. First, it is highly unlikely that the preference is there only in the third week. This is probably an artifact of the motor skill demands of our experimental set-up. Hepper (1987a), immediately after birth, and a number of other authors (reviewed in Chapter 2), have shown in a variety of experiments from different theoretical perspectives that some form of preference exists earlier.
Second, the significance of our experiments lies in the fact that a set-up is chosen, which imitates natural circumstances while, of course, excluding the possibility of interference from animals other than the experimental subject. The stimulus complex is diverse, with odors emanating from different nests and from both the mothers and the litter-mates. Also, the period that is encompassed in our experiments is the period that the pre-weanling rat is motorically able to stray away from the nest, while still dependent on its resources for food, shelter and warmth. In the fourth week, rats start to become self-reliant and activation of the attachment behavioral system is then no longer necessary.
Third, it is not our intention to equate attachment in rats with attachment in humans. The manifestation of attachment in different species is influenced by natural selection along the lines of the demands created by the social organization of the species. Our contention is that there are relevant similarities in the neurobiological substrate, but that they are not identical and neither are the behaviors.

Kin recognition
In the discussion in Chapter 3, some attention is devoted to the demarcation between kin selection and attachment theory, which results in the second experiment of Chapter 4. In the literature on genetic relatedness and familiarity in preference experiments, the

terminological problems were striking. Although several authors have argued for terminological purity and scientific caution, this was to no avail. An important cause lies in the dominance of the kin selection paradigm (Hamilton, 1964) in ethology. This dominance has led to a widespread and problematic use of the term 'kin recognition'. Byers & Bekoff (1986) discuss two meanings of the term kin recognition. The first is the 'unobservable neural process', the mechanism underlying the behavior. The second refers to the overt behavior, i.e. 'differential treatment of kin'. Byers & Bekoff preferred the use of the first form, if the term recognition were to be used at all. The manifest behavior should then be referred to as kin-correlated/kin-aligned behavior. Waldman, Frumhoff and Sherman (1988) and Hepper (1991 & 1999) shared this preference for referral to mechanism, but preferred the term 'kin discrimination' for the observable behavior. On the other hand, Holmes and Sherman (1983) define kin recognition as 'differential treatment of conspecifics (including the self) differing in genetic relatedness'. This definition according to observable behavior was reiterated by Sherman et al. in 1997. Thus, Sherman first supported the behavioral meaning, then the mechanism meaning and finally again the behavioral. This struggle is reflected by the definition given by Pfennig (2002): "Kin recognition is the ability to identify relatives. More precisely, it is the differential treatment of members of the same species in a way that depends on their genetic relatedness to the discriminating individual." Given these two meanings, a third way of defining kin recognition was unwelcome. Yet Stuart (1991) wrote: 'recognition systems...may well function to associate kin in a fitness enhancing concept'. This definition of 'kin recognition as a functional concept' explicitly introduces the supposed ultimate consequences that are more implicitly present throughout the literature. A fourth terminological suggestion is found in Erhart et al. (1997). They distinguish kin recognition from kin discrimination in the following way: '*kin recognition* is defined as the ability to identify or categorize conspecific individuals as either kin or non-kin. This contrasts with *kin discrimination*, which refers to the ability to recognize various categories of relatives (e.g. full-sibling vs. half-sibling or aunt vs. grandmother).' The use of the term ability is especially confusing, because it is unclear if this refers to properties of the animal or expression of these properties in an experiment, i.e. outcome. In addition, apart from a fourth meaning of the term kin recognition they, also introduce a second meaning of the term kin discrimination. Sometimes the semantic confusion generated by the term kin

recognition is evaded altogether, as in the review by Brown and Eklund (1994), who state 'we use the term kin recognition in its conventional sense' without explaining which conventional sense that is.

Byers & Bekoff (1986) advocate a clear terminological distinction between mechanism and behavior because a lack of it leads to 'fallacies associated with arbitrary inference concerning processes about which we know little or nothing'. This is, of course, justified, but we argue that it is also necessary to make a clear distinction between behavior and outcome in discussing experimental results. Indiscriminate use of the term behavior can lead to generalizations, namely, that the behavior in a specific experiment is the behavior that the species will demonstrate in other experiments, or even in their natural environment. Subsequently, it becomes more tempting to make inferences about mechanisms underlying the behavior. This process of generalization and unjustified inference can be found in the exchange between Grafen and others about Grafen's strict interpretation of kin recognition as 'genetic similarity detection'. Many outcomes of kin-recognition experiments are discussed, and according to these outcomes, terms are used such as 'true kin recognition system' (Grafen 1990), 'littermate recognition system' (Grafen 1991) and 'group or individual recognition systems' (Stuart 1991). Another example of this two-step generalization from outcome via behavior to mechanism is found in Hepper (1999), when he discusses experiments in domestic dogs. The outcome of these experiments was that dog pups at 4-51/2 weeks of age did 'recognize' their mothers and their siblings. After 2 years of separation, these dogs still showed differential behavior to their mother, but not to their siblings. Hepper then concludes that 'this indicates that...different mechanisms underlie the ability of an individual to recognize its mothers and its siblings'. If the period of separation had been 2 months instead of 2 years, and both mother and siblings had been recognized, would this have meant that there was only one mechanism underlying the behavior? Kruczek (2007) presents a comparable example in suggesting the existence of two kin-recognition mechanisms in discussing an experiment in female bank voles (Clethrionomys glareolus). They showed a preference for familiar male age-mates before weaning and for unrelated age-mates after weaning. Change in the decision rules leading to a change in behavior during development does not mean that there are two qualitatively different mechanisms of recognition that precede the behavior.

These examples illustrate the necessity of a sophisticated use of terminology: outcome is dependent on choice of species (e.g. precocial versus altricial), past developmental experiences (e.g. cross-fostering or separation procedures), present environmental influences (e.g. diet) and last but not least, choice of dependent variables (familiar vs. non-familiar, siblings vs. mothers etc.). Hence, outcome cannot be generalized to behavior, nor behavior to an underlying mechanism, nor a hypothetical underlying mechanism to evolutionary advantages.
The conclusion about the term kin recognition must be that until now it has had more costs than benefits:

- Its definition is variable, with the confusion between behavior and mechanism inadvertently leading to inferences about mechanisms based on outcome. Outcomes have never until now been more than the expression of some kind of familiarity assessment mechanism, (although sometimes self-referencing) and the use of the term kin recognition has obscured this;
-The term kin recognition automatically evokes the term kin selection, leading to more or less implicit generalizations about ultimate functions of experimental outcomes. Alternative ultimate explanations, for instance according to attachment theory, are then ignored;
-It is used in articles that describe experimental discrimination of: a. kin and non-kin of the subject (e.g. Hepper 1983 & 1987b, Wills et al.,, 1983); b. kin of familiar non-kin and totally unrelated animals (Porter 1986, Hepper, 1991); c. kin relations between unfamiliar conspecifics (Parr & de Waal, 1999, Porter, 1999); d. kin relations between members of a totally different species (see the article by Ables et al., 2007 on how rats can distinguish kin relations between humans).

This shows the confusing realm of phenomena to which it refers, most of which have no functional, let al.one selective significance.

The role of oxytocin and vasopressin

Data concerning oxytocin gathered from different areas of attachment-related research largely point in the same direction. In humans, blood oxytocin levels are positively correlated to positive social behavior (Uvnas-Moberg et al., 1991, Zak et al., 2005, Light et al., 2005) and intranasal administration increases positive behavior (Kosfeld et al., 2005, Zak et al., 2007) and recognition of positive facial expressions (DiSimplicio et al., 2008). Focusing on attachment *sensu stricto*,

comparable results are found. Bonding representations to the parents are positively correlated to oxytocin in adults (Gordon et al., 2008) and plasma oxytocin levels during pregnancy to indices of post-natal, maternal-infant bonding (Feldman et al., 2007). Activation of positive and negative emotions, however, does not lead to a corresponding rise and fall in oxytocin levels (Turner et al., 2002). Early attachment experiences seem to be reflected in oxytocin levels, both in humans and in monkeys. The rise in oxytocin levels during a positive interaction with their mother, in comparison with a similar interaction with an unknown female, was greater in control children than in children with an orphanage history, even after an average of three years in a normal family (Fries et al., 2005). In monkeys, Winslow et al. (2003) showed that oxytocin concentrations in cerebrospinal fluid were reduced in nursery-raised rhesus males in comparison with mother- raised monkeys. In rodents, oxytocin promotes the formation of pair bonds in female prairie voles (see review by Lim and Young, 2006). In rats, Nelson and Panksepp (1996) showed that facilitation of odor learning in the presence of the mother was blocked in rat pups by an oxytocin antagonist. Our own results (see Chapter 4) were negative: oxytocin did not influence attachment behavior.
However, it would be unjustified to say that the oxytocin results are unequivocal. First, some of the experiments mentioned above are concerned with positive social behavior and not with attachment behavior in particular. Second, the orphanage and nursery research question the direction of causation: in these studies positive attachment experiences seem to promote oxytocin instead of the other way around. Third, there are the conflicting results of the studies on the influence of oxytocin on ultrasonic distress vocalizations in rat pups. Peripheral administration has a biphasic effect on USVs, while central administration reduces USVs (Insel and Winslow, 1991). This implies that the presence of oxytocin reduces USVs, but oxytocin knock-out (Winslow et al., 2000) and oxytocin receptor knock-out (Takayanagi et al., 2005) mice vocalize less, implying that the absence of oxytocin reduces USVs. Part of the problem here is that ultrasonic distress vocalizations are a complex behavior involving different systems. USVs are emitted when the organism experiences stress, so stress-reducing hormones like oxytocin should reduce them. However, they are also a form of attachment behavior, calling the care-giver. A substance that promotes attachment behavior, as oxytocin has been shown to be in numerous experiments, should increase USVs. On the other hand, in a goal-corrected behavioral system, it is possible that

oxytocin switches on the behavior but that above certain levels, it switches the behavior off again. Thus, USV research is not very helpful in establishing the role of oxytocin in attachment. Yet the conclusion is that oxytocin generally is positively correlated to attachment behavior.
The situation is much less clear with regard to vasopressin. The results in USVs are as complex as in oxytocin: central vasopressin-reduced vocalizations, the effect of which was blocked by a V1a receptor antagonist, that could not block the increase of vocalizations caused by peripheral administration (Winslow and Insel 1993a). Ijima & Chaki (2005) and Hodgson (2007) both report that a V1a-antagonist reduces USVs. Given the role of vasopressin in the stimulation of the stress-response (Caldwell et al., 2008), the reducing effect of the V1a-antagonist is understandable, but the earlier results are hard to interpret. As mentioned above, this can also be due to the complexity of USVs. In male prairie voles, vasopressin has the same function as oxytocin in female prairie voles (Winslow et al., 1993b): promoting pair-bond formation, an effect that is blocked by an antagonist. We showed a reduction in attachment behavior in female rat pups by vasopressin (see Chapter 4), which is as yet an isolated finding. In humans, a very recent study showed an association between a polymorphism of the V1a receptor antagonist and measures of marital stability (Walum et al., 2008). In conclusion, there is much less research done on the relationship between attachment and vasopressin than on the relationship with oxytocin. The multitude of functions that vasopressin has in pair bonding, stress, aggression, memory, etcetera, makes it very difficult to design experiments that isolate one of these functions.

The relationship between early attachment and adult behavior
Extra face validity of the rat as an animal model for infant-mother attachment was the reason for investigating whether it was possible to induce pair bonding in adult rats. In natural circumstances, the rat is polygamous. However, if it were possible to induce partner preference experimentally, the rat's normal polygamous behavior can be considered a consequence of ecological circumstances. The plausibility of the existence of a "dormant" neurobiological substrate, which is an evolutionary precursor of the one responsible for attachment behavior in humans, would be increased. Because of the great interest created by the research on pair bonding in prairie voles, a comparable experimental procedure was chosen (Winslow et al.,

1993b). But where prairie voles had to choose between the previous cohabitation partner and one unfamiliar other, we used the four-way choice model we had used earlier in pups. The fact that we could induce a partner preference in male rats through a 24-hour cohabitation period was, of course, encouraging for the model. The fact that this preference disappeared after careful removal of olfactory stimuli, which could have come from the male, was a surprise. We have never seen any mention of the removal of these remnants in the literature on voles. As mentioned in Chapter 5, this creates the possibility that "partner preference" in the literature on voles is nothing other than the recognition of familiar, self-referent stimuli, for instance traces of urine. In contrast to many other species, prairie voles engage in scent-marking their environment and their conspecifics with urine (Mech et al., 2003) as do rats (Taylor et al., 1984). In this line of thinking, partner preference or adult attachment is a later evolvement of a neurobiological system that might originally have had a territorial function. To speculate even further: neuropeptide effects on development and blockade of partner preference in prairie voles could have been purely cognitive in nature, e.g. changing alertness, discrimination between stimuli or memory function. The role of neuropeptides where cognition and social behavior interface, namely in social recognition, is well known (see Bielsky and Young, 2004, for a review). In any event, in future research into attachment, careful delineation of these different possibilities is of importance.
In Chapter 6 we report on the effects of attachment disturbances by means of a (twice) daily cross-fostering intervention during infancy on adult behavior. There are various reasons why we consider the results meaningful. First, as stated in Chapter 6, one of the two criteria for an animal model of attachment is that response to removal of the attachment object is distinct from the response to reduction in social stimuli *per se*. Our experiment was an extreme interpretation of this criterion, namely not the removal of the attachment object, but actual prevention of the development of attachment altogether by frequent cross-fostering. The 'response to removal of the attachment object' was recorded much later, namely in adulthood. Although there is room for debate as to whether our experiment suits the letter of the criterion, the essence of the experiment was that the amount of social stimuli was identical between the two groups. Only the presence of a consistent attachment object differed and therefore we argue that it was at least in the spirit of the criterion.

Second, we were interested in a replication of the findings in humans with regard to the negative effects of early attachment disturbances on adult functioning. The review in Chapter 1 of the relationship between attachment development and psychopathology clearly illustrates this connection and it would strengthen the model if we could find comparable results in rats. We think that the results presented in Chapter 6 are indeed a confirmation of the hypothesis that the normal development of attachment-related processes is essential for normal psychosocial and psychosexual behavior in adulthood in both humans and rats.
Third, on a precautionary note that this is highly speculative, our results do not conflict with the literature mentioned in the paragraph on oxytocin in this general discussion. Studies on neglected children (Fries et al.,. 2005) and on nursery-raised monkeys (Winslow et al., 2003) both showed a reduction in oxytocin levels as a consequence of disturbed early attachment. The behavioral differences we found were increased anxiety-like behavior and passive coping in the elevated plus maze and the shock-prod bury test respectively and increased latency to engage in sexual behavior. Oxytocin has a role in dampening the stress response (Neumann, 2008) and is important for sexual behavior in male rats (Melis et al., 2007). The fact that these were both disturbed as a result of our intervention does not conflict with a negative influence of attachment disturbance on oxytocin levels in rats.

Evolutionary perspective
The evolutionary perspective that is so prominent in Bowlby's thinking has hardly been discussed until now. Because natural selection exerts its effects over multitudes of generations, research into it is exceedingly demanding and time-consuming and has not been performed on attachment. However, the evolutionary perspective is one of the most appealing features of attachment theory, distinguishing it from other behavioral theories and opening up the possibility for research on lower animals as in this thesis. Central to attachment theory is the notion that, during the course of evolution, selective advantages have been acquired by organisms carrying genes, which contribute to the functioning of the attachment behavioral system (Bowlby, 1969). These selective advantages are the result of a strong bond between an infant and its care-giver in the course of evolution, usually the mother. The relationship between the infant and the mother is bi-directional in nature and both parties benefit from it.

In natural circumstances the inclination of a child to attach to a specific care-giver has the advantage that care-giving behavior is elicited from an individual with a strong genetic relatedness, namely an r of 0.5. The care-giver promotes the survival of an individual with half of the care-giver's genes, so care-giving behavior has selective advantages. For the infant it seems relatively unimportant if the essential food for survival and protection are procured by a genetically-unrelated or a genetically-related individual. This is reflected in the fact that the infant forms attachment relationships with individuals who are not genetically related as easily as with a parent. The specificity of the relationship, however, seems a necessary pre-condition. If the infant does not behave in a way that leads to a relationship with a specific other, it is not very likely that a specific individual will feel responsible for answering the infant's call for nurturing or protection. This will reduce the possibility that anyone at all will respond to the call or will feel responsible to stay in the vicinity of the infant in case the infant needs something. The advantage of the specificity for the care-giver is, as mentioned above, that in natural circumstances, the infant carries half of the care-giver's genes. Unspecified care-giving behavior has the effect that resources are also used by unrelated infants, leading to less selective gain. Maternal selectivity of care-giving behavior towards the mother's own children is, of course, strongly reinforced by the process of pregnancy and parturition. During pregnancy and the first post-natal period, procreation is not possible for the maternal figure and this increases the selective advantage of caring for the newborn as a method of promoting survival of (half of) the genes. The paternal figure, however, has alternative methods of fostering the interests of his genes during this period, namely procreation with other females. This is probably part of the explanation as to why the attachment relationship with a biological child is less strong in males than in females.

Conclusion

To conclude, this thesis shows that attachment theory has many implications that have found support in scientific research. It seems indisputable that disturbed attachment has a causative relationship with psychopathology. From the research carried out on rodents for a number of reasons, the conclusion can be drawn that rats do tend to develop a preference for a specific care-giver which, in natural circumstances, is the mother. Our experiments confirm this

development and describe it more precisely in an experimental design that creates a stimulus environment that is more ecologically valid than is usually the case in preference experiments on rodents. The most common alternative explanation for an attachment-based preference in rat pups for the home environment, namely a preference for genetically-related animals, is unconfirmed. This strengthens the rat as an animal model for infant-mother attachment. The experimental design lends itself to pharmacological interventions. Further support for the animal model is gathered by the negative effects on adult behavior of an attachment-preventing, cross-fostering intervention during infancy. Additional evidence comes from the replication of partner preference-inducing procedures derived from research on prairie voles, the most prominent model for monogamy, i.e. adult attachment. The rat is suitable for research into the behavioral, endocrinological and anatomical aspects of the neurobiological substrate of attachment behavior.

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Samenvatting.

Dit proefschrift is gericht op het onderzoeken van de mogelijkheid dat de rat kan worden gebruikt als diermodel voor hechting. De hechtingstheorie van Bowlby is momenteel de enige ontwikkelingspsychologische theorie die kan bogen op een degelijke wetenschappelijke onderbouwing. Nochtans wordt de hechtingstheorie in de dagelijkse klinisch-psychiatrische praktijk nog weinig gebruikt. Dit is mede te wijten aan het feit dat, zeker nu de psychiatrie in de afgelopen decennia een sterk biologisch karakter heeft gehad, onvoldoende bekend is over de biologische basis van hechtingsgedrag. Het is nog grotendeels onduidelijk welke hersenstructuren of hormonale (hersen)processen ten grondslag liggen aan de neiging om een gehechtheidsrelatie aan te gaan met specifieke personen en toenadering te zoeken tot die personen.in geval van stress, angst of vermoeidheid. Om de biologie van hechting te bestuderen is het nodig een goed diermodel te hebben waarbij dit onderzoek gedaan kan worden. Experimenteel onderzoek bij mensen stuit immers al snel op bezwaren. Ook onderzoek bij apen is om praktische en ethische redenen vaak ingewikkeld. Vandaar dat het nuttig zou zijn als de rat als diermodel voor onderzoek naar hechting gebruikt zou kunnen worden. De rat biedt een aantal voordelen: ratten zijn makkelijk en goedkoop te houden, ze krijgen nesten die voldoende groot zijn om binnen één nest verschillende interventies te doen bij zowel mannetjes als vrouwtjespups en er is al veel over de fysiologie en het gedrag van de rat bekend.

In **hoofdstuk 1** wordt een overzicht gegeven van de geschiedenis van de hechtingstheorie. Dit wordt gedaan aan de hand van drie belangrijke personen uit die geschiedenis: John Bowlby, Mary Ainsworth en Mary Main. Vervolgens worden een aantal stellingen besproken die logischerwijs voortvloeien uit het gedachtengoed van de hechtingstheorie. Er wordt aandacht besteed aan het wetenschappelijk bewijs wat bestaat voor: de veronderstelde aanwezigheid van een specifiek neurobiologisch substraat; de -gezien de focus op reële ervaringen- relatief geringe invloed van genetische factoren en de aanwezigheid van verschillende gehechtheidsrelaties met verschillende personen; de aanwezigheid van de overdracht van gehechtheidspatronen van de ene generatie op de andere, bij voorkeur ten gevolge van empathie; de veronderstelling dat psychopathologie meer voorkomt bij onveilig gehechte mensen; de veronderstelling dat

gehechtheidspatronen na de vorming ervan in de eerste levensjaren relatief stabiel zijn, maar toch gevoelig voor een consequente verandering in hechtingscontext. Vervolgens wordt een overzicht gegeven van meetinstrumenten voor hechting bij de mens en de relatie tussen verstoorde hechting en psychopathologie. De conclusie is dat psychopathologie meer voorkomt bij onveilig gehechte mensen. De zgn. gedesorganiseerde gehechtheidsstijl komt vaker voor bij kinderen met zowel internaliserende (angst, depressie en lichamelijk beleefde klachten) problematiek als externaliserende (gedragsproblemen) problematiek. Bij volwassenen heeft deze gehechtheidsstijl een relatie met traumatische stoornissen en borderline persoonlijkheidsproblematiek. Gepreoccupeerde gehechtheid is gecorreleerd met angst en stemmingsproblemen bij kinderen en met angst, depressie, eetstoornissen, somatisatie en borderline problematiek bij volwassenen. Er is een onduidelijke relatie tussen vermijdende gehechtheid en psychopathologie, hoewel er wel aanwijzingen zijn dat vermijdend gehechte personen geneigd zijn tot onderrapportage van problemen in zijn algemeenheid. Verder wordt de band met de ouders over het al.gemeen als minder goed beleefd bij mensen met psychiatrische problematiek. Zowel gebrek aan ouderlijke zorg als ouderlijke overbescherming zijn gerelateerd met eetstoornissen, depressie, schizofrenie en persoonlijkheidsproblematiek.

In **hoofdstuk 2** wordt bekeken in hoeverre experimenten waarbij knaagdieren een voorkeur moeten tonen in sociale situaties opnieuw geïnterpreteerd kunnen worden vanuit een hechtingskader. Die experimenten zijn over het al.gemeen niet vanuit zo'n kader gedaan, maar vanuit belangstelling voor de ontwikkeling van zintuigen of motoriek, het stress-systeem of de ontwikkeling van leren. Deze experimenten laten zien dat infantiele knaagdieren al vanaf vlak na de geboorte, of wellicht zelfs voor de geboorte, in staat zijn om met behulp van hun reukvermogen hun moeder te onderscheiden van andere soortgenoten en er ook een voorkeur voor hebben. Ook kunnen prepubertale ratten een voorkeur voor allerlei stimuli ontwikkelen als ze worden aangeboden in aanwezigheid van de moeder. Ook andere soortgenoten dan de moeder kunnen de voorkeur krijgen als ze warmte of voeding leveren, maar een dergelijke voorkeur is minder sterk dan voor de moeder. Farmacologisch gezien is het vooral oxytocine wat een positieve invloed heeft op hechtingsgedrag c.q. de ontwikkeling van voorkeur voor een specifieke partner. Bij de

prairiewoelmuis heeft oxytocine bij vrouwtjes een positieve invloed op de ontwikkeling van monogamie en vasopressine bij mannetjes.
In **hoofdstuk 3** wordt het eerste eigen onderzoek gepresenteerd. Hierbij wordt jonge ratten een keuze geboden tussen twee nesten. Rattenpups worden één voor één in een centraal gebied tussen twee compartimenten met nesten geplaatst waarvan ze gescheiden worden door kippengaas. Hierdoor is het niet mogelijk voor de moeder om te interveniëren. Het centrale gebied waarin de pup zich bevindt wordt verdeeld in een gebied wat grenst aan het nest waar ze zelf uit afkomstig zijn en een gebied wat grenst aan een nest wat eveneens bijvoorbeeld twee weken eerder ter wereld is gekomen. Een boven het centrale gebied hangende camera filmt de rattenpup en via een computer wordt automatisch geregistreerd hoe lang de pup in de verschillende gebieden doorbrengt. Er blijkt zich een duidelijke voorkeur voor het thuisnest te ontwikkelen inde loop van de tweede week na de geboorte die zijn hoogtepunt bereikt op dag 17 en daarna weer afneemt. Tijdens een test van 900 seconden brengt de rat op dag 17 ongeveer 2/3e van de tijd door aan de kant van zijn thuisnest. Met controle-experimenten is vervolgens gekeken of er verschil is in de voorkeur op dag 17 en op dag 24 tussen pups die vanaf dag 12 dagelijks worden getest en pups die slechts eenmalig worden getest. Dit blijkt niet het geval.
In **hoofdstuk 4** wordt een verbeterde versie van de onderzoeksopzet uit hoofdstuk 2 gepresenteerd. Hierbij moet de rattenpup niet kiezen tussen twee maar tussen vier compartimenten met elk 1 moeder met haar nakomelingen. Met behulp van een pupverwisselingsexperiment wordt onderzocht waardoor de voorkeur tot stand komt. De helft van de pups werd op dag 1 na de geboorte in een ander nest geplaatst en later werd de mogelijkheid geboden om te kiezen tussen het 'adoptienest', het nest waar ze in ter wereld waren gekomen en twee nesten waar ze noch in waren opgegroeid, noch genetisch aan verwant waren. Is het zo dat pups in staat zijn om op grond van geur nesten te herkennen als zijnde genetisch verwant of moeten ze gedurende de ontwikkeling contact hebben gehad met dat nest om daar een voorkeur voor te hebben? Dit laatste blijkt het geval. Rattenpups maken geen onderscheid tussen een nest waar ze zelf in geboren zijn en dus genetisch mee verwant-maar waar ze zelf niet in zijn opgegroeid- en nesten waar ze ook geen ervaring mee hebben maar die daarbij genetisch niet verwant zijn. Dit resultaat wordt besproken in het licht van de kin selectie theorie van Hamilton. Deze theorie is erg dominant in de ethologie en bepaalt het denken over voorkeursexperimentent

veelal, maar eigenlijk is het nog onduidelijk wat bij knaagdieren het evolutionaire voordeel van het herkennen van genetisch verwante dieren zou kunnen zijn. De evolutionaire voordelen van het ontwikkelen van een voorkeur voor een dier waarvan de ervaring heeft geleerd dat het verzorging en bescherming biedt zijn evident. Tenslotte worden in hoofdstuk 4 experimenten met oxytocine en vasopressine getoond. Pups werden vlak voor ze in de testopstelling een voorkeur voor een thuisnest konden laten zien met deze farmaca ingespoten. Er bleek geen significant effect van oxytocine op te treden, terwijl vasopressine de voorkeur voor thuis verminderde.
In **hoofdstuk 5** werd gekeken of het mogelijk was bij volwassen mannelijke ratten een voorkeur voor een partner tot stand te brengen. Er werd een protocol gevolgd dat ook bij de prairiewoelmuis wordt gehanteerd, de diersoort die het meest gebruikt wordt bij het onderzoek naar partner preferentie. De rat werd gedurende 24 uur samen in een kooi gezet met een vrouwtje en later moest het mannetje kiezen tussen 4 vrouwtjes waarvan 1 de partner was. Ratten bleken, ondanks het feit dat ze in natuurlijke omstandigheden polygaam zijn, door de 24 uur van samen zijn een voorkeur voor dat vrouwtje te ontwikkelen, dat wil zeggen dat ze meer tijd doorbrachten aan de kant van dat vrouwtje dan aan de kant van één van de drie onbekende vrouwtjes. Ook het gedurende 2 in plaats van 24 uur samen zetten van een mannetje met een vrouwtje leidde tot een partner voorkeur. Echter, als er voor gezorgd werd dat er zich in het zaagsel in de kooi van het vrouwtje of in de vacht (door te wassen met shampoo) van het vrouwtje geen geursporen van het mannetje kon bevinden verdween de voorkeur. Dit opent de interessante mogelijkheid dat partnervoorkeur het gevolg is van het herkennen van eigen stimuli, dus in feite een soort territoriumdrift is.
In **hoofdstuk 6** werd opnieuw gebruik gemaakt van pupverwisseling. In de eerste 20 dagen van het leven van de pups werd de helft dagelijks in een ander nest geplaatst en in een tweede experiment zelfs twee keer per dag. Er werd zorgvuldig op toegezien dat er tussen de verwisselde en de niet verwisselde pups geen verschillen waren in de tijd waarin ze van het moederdier en de nestgenoten waren gescheiden of in de hoeveelheid hantering c.q. aanraking die ze moesten ondergaan. Dat wil zeggen dat niet verwisselde pups ook iedere dag werden opgepakt, apart gezet en even later weer teruggezet, maar dan gewoon weer bij hun eigen moeder. De bedoeling van deze experimenten was om door de veelvuldige wisseling bij de ene helft te voorkomen dat de pups een hechtingsrelatie konden ontwikkelen met

één specifieke moeder. Vervolgens werd in de volwassenheid gekeken of dit voorkomen van hechting effect had op het gedrag. Daartoe werden de dieren aan een aantal experimenten onderworpen. Samenvattend kan gezegd worden dat voorkóming van hechting in de jeugd in de volwassenheid leidde tot een toename van angstig en passief gedrag en een vertraagde afloop van seksueel gedrag.
Hoofdstuk 7 bevat een algemene discussie over het proefschrift. Er wordt geconcludeerd dat het nodig is om de hechtingstheorie een betere biologische onderbouwing te geven. Dit is wenselijk omdat de rol van hechting bij psychiatrische problematiek zo evident is dat de patiënten te kort wordt gedaan als de hechtingstheorie niet meer ingang vindt in het klinische werk. Ook wordt geconcludeerd dat de rat goede perspectieven biedt om als diermodel te fungeren. De rat ontwikkelt een duidelijke voorkeur tijdens de jeugd voor de thuiskooi en die voorkeur is farmacologisch te beïnvloeden. Ook is het zo dat de volwassen rat, zoals de prairiewoelmuis en de mens, in staat is om een voorkeur te ontwikkelen voor een specifieke partner. Tenslotte is het zo dat ook bij de rat verstoring van de hechting gedurende de ontwikkeling leidt tot afwijkend gedrag in de volwassenheid.

Curriculum vitae.

Heinrich Otto Sigling werd op 20 juni 1956 geboren in Amstelveen. Hij behaalde het Gymnasium-β diploma aan het Hermann Wesselink College aldaar in 1975. In dat jaar begon hij de studie geneeskunde aan de Vrije Universiteit te Amsterdam. Tijdens de studie was hij student-assistent bij de vakgroep Histologie en deed onderzoek naar het Bronchus Associated Lymphoid Tissue van de kip. In 1984 studeerde hij af. Zijn eerste baan als arts was in 1985 bij het Luthers Diakonessenhuis te Amsterdam, als arts voor nacht- en weekenddiensten voor alle specialismen. In oktober 1985 begon hij als verslavingsarts in de hiërarchische therapeutische gemeenschap het Oolgaardthuis te Arnhem, onderdeel van APZ Wolfheze, en was daar behandelcoördinator van de Detox. Daarna werkte hij bij het APZ Bloemendaal te Den Haag en bij het PC St. Willebrord te Heiloo als arts-assistent psychiatrie en bij de Riagg Rivierenland te Tiel als behandelcoördinator van het team Volwassenenzorg. Gedurende een half jaar volgde hij de opleiding tot systeempsychotherapeut bij de RINO Amsterdam, maar werd toen aangenomen bij de opleiding tot psychiater in het Radboudziekenhuis te Nijmegen. Aanvankelijk was prof. G.J. Zwanikken daar opleider, later Prof. F.G. Zitman. In het kader van de opleiding werkte hij in de Psychiatrische Universiteits Kliniek, de polikliniek kinder- en jeugdpsychiatrie, de psychiatrische consultatieve dienst van het Radboudziekenhuis, de polikliniek Volwassenen van het APZ Groot-Graffel te Warnsveld en bij de Riagg Noord-West Noord Brabant te Oss. De opleiding werd afgesloten met een préklinische onderzoeksstage van een half jaar bij het Rudolf Magnus Instituut voor Farmacologie van de faculteit Geneeskunde van de Universiteit van Utrecht, waarvan prof. W.H. Gispen toen directeur was.
Na afloop van de opleiding bleef hij bij het RMI onderzoek doen, bij de groep van Dr. B.M. Spruijt. In juli 1995 werd hij tevens psychiater bij de buitenkliniek Vosseveld te Soest, onderdeel van de afdeling Kinder- en Jeugdpsychiatrie van het Academisch Ziekenhuis Utrecht, die toen werd geleid door prof. dr. H. van Engeland. Tijdens die aanstelling werd ook de registratie als Kinder- en Jeugdpsychiater verworven. In de periode bij het AZU, die duurde tot 1 januari 2001, was hij gedurende 5 jaar voor 1 dag per week gedetacheerd bij het Regionaal Diagnostisch Team van de Jeugdhulpverlening bij de Riagg

Stad Utrecht. Van april 2001 tot april 2002 was hij content redacteur van een website voor werkende moeders. Van februari 2002 tot april 2006 was hij 1 dag per week als psychiater verbonden aan de HSK-groep, een bureau voor arbeidsgerelateerde psychische problematiek. In 2002 werkte hij een half jaar als waarnemer bij het team Alkmaar Centrum van de Dijk, afdeling voor chronische psychiatrie van de GGZ Noord Holland Noord. Van november 2002 tot april 2006 was hij Manager Zorg van het circuit Aanmelding, Reclassering en Activering van de Jellinekkliniek te Amsterdam. Sinds april 2006 werkt hij voor de Brijder Verslavingzorg, onderdeel van de Parnassia Bavo Groep. Hij is psychiater van het team Zaandam, hoofd behandeling van de klinische afdelingen en geneesheer-directeur. Hij woont met vriendin en drie kinderen in Amstelveen.

Dankwoord.

Nog geen 15 jaar geleden, op 1 april 1994, begon ik in het net van de Vondelstraat naar de Uithof verhuisde Rudolf Magnus Instituut aan het onderzoek dat heeft geresulteerd in dit proefschrift. Veel mensen hebben in woord of gebaar iets bijgedragen aan de totstandkoming ervan. Vijftien jaar is lang en niet alles en iedereen staat mij nog helemaal helder voor de geest. Wie vind dat hij/zij ten onrechte ontbreekt in de nu volgende opsomming heeft gelijk en slechts de tand des tijds is er verantwoordelijk voor. Ook degenen die in mijn professionele leven op een andere manier dan voor dit proefschrift van belang of leuk zijn geweest komen in dit dankwoord niet voor. Het zijn er teveel.

Willem-Hendrik Gispen en Berry Spruijt, mijn promotoren, hebben mij van het begin af aan met open armen ontvangen. Slechts nu en dan, en uitsluitend volkomen terecht, werd ik gewezen op het feit dat ik slechts psychiater was en derhalve begrijpelijkerwijze niet op de hoogte van de meest elementaire principes van préklinisch onderzoek. Naast de open armen stelde ik ook de grote vrijheid op prijs die mij ten deel viel, zowel met betrekking tot de inhoud van mijn onderzoek als het tempo waarmee ik de data omzette in hoofdstukken.

Herman van Engeland speelde aanvankelijk ook een rol als promotor. Door onenigheid over klinische kwesties kwam daaraan een eind. Nochtans wil ik hem bedanken voor de mogelijkheid die hij me gaf in zijn afdeling Kinder- en jeugdpsychiatrie aan de slag te gaan.

Ook anderen hebben mij in de afgelopen 15 jaar in de gelegenheid gesteld bent om in mijn levensonderhoud te voorzien terwijl ik dus intussen in feite met niets anders bezig was dan dit proefschrift: dank voor de ruimte! Het gaat behalve om de buitenkliniek Vosseveld in Soest om Motheraffairs b.v., HSK, de GGZ Noord-Holland Noord, de Jellinek en Brijder verslavingszorg.

De weg naar het onderzoek, met een begin als keuzestage tijdens de psychiatrie-opleiding, is voor mij geplaveid door drie eminente mannen. Als eerste noem ik David de Wied, in leven tot zijn pensionering hoogleraar Farmacologie in Utrecht en directeur van het Rudolf Magnus Instituut. Ik kwam bij hem over de vloer om

privéredenen. Daar ontdekte ik dat wetenschap een activiteit kan zijn met glamour en buitenlandse reizen en dat er met wetenschappers soms veel te lachen valt. Hierdoor werd wetenschappelijk werk een serieuze optie. Jaren later, op het RMI, spraken we nogal eens over mijn onderzoek en ook schreven we samen een artikel. Hij was uitzonderlijk scherpzinnig, kritisch en direct, maar bovenal geestig. Henk Vlaar, zenuwarts/psychoanalyticus, is van 1985 tot 1992 mijn therapeut geweest. Zonder hem had ik de jacht op een opleidingsplaats niet 5 jaar volgehouden, had ik nooit voor een préklinische keuzestage durven kiezen en had ik dit proefschrift niet afgemaakt. Naast veel andere dingen leerde hij mij dus te doen wat ik zelf de moeite waard vind. Daarnaast is hij veruit mijn belangrijkste rolmodel voor wat betreft de attitude tegenover patiënten.
De enige nog levende van de drie mannen zonder wie dit proefschrift er zeker niet geweest was is Goos Zwanikken. Hij was tot 1992 hoogleraar psychiatrie aan de Katholieke Universiteit Nijmegen. Hij nam mij, in een tijdperk dat er vele kandidaten voor één plaats waren en ik al bijna in het hele land was afgewezen, aan voor de opleiding tot psychiater. Hij wees ons op het belang van gewóón doen en was een charismatische leider met een zeer effectief soort milde onverbiddelijkheid die je op een management-opleiding niet snel zult leren.

Eenmaal op het RMI aangekomen maakte ik deel uit van de gedragsgroep van Berry Spruijt. In de werkgroepbesprekingen waar talloze experimenten van studenten en AIO's de revue passeerden heb ik geleerd wat goede en slechte wetenschap is en is mijn onderzoek vaak in de juiste richting bijgestuurd. Jacob, Thorwald, Inge, Josefien, Carolien, Leon, Hans, Patrick en Robert waren collega onderzoekers in de eerste jaren. Het was een gezellige club waar ook nog wel eens aandacht werd besteed aan wijn en bier.

Onderzoek doen is behalve een gespreksonderwerp ook een bezigheid. Daar komen apparaten bij te pas, en dieren en technieken. Marlou Josephy en vooral Annemarie Baars hebben mij ingewijd in het omgaan met ratten, camera's en computerprogramma's. Later kwam Inge Wolterink erbij. Zij heeft ook veel experimenteel werk gedaan. Annemarie en Inge waren erg aardig en steunend, maar ze hebben ook een enorme hoeveelheid kennis en ervaring en zijn van onschatbare waarde voor onderzoekers. Zij stuurden ook de studenten aan die een deel van het hier beschreven werk voor hun rekening namen. Ik dank

vooral Iolente Korstjens, Noranne Langerak, Sander Kappen en Mijke Kruidenier.

De ratten die in de beschreven experimenten gebruikt werden moesten natuurlijk worden verzorgd: voor, tijdens en na de experimenten. Vooral John de Jong, Bert Tersteeg en Wout Puijk hebben dat gedaan in de periode dat ik onderzoek deed. Behalve voor dierverzorging kon ik achter de barrière ook terecht voor koffie en grappen.

De voormalige directeur sociale zaken van het RMI, Ton van den Brink, is de man die de op de voorkant van dit proefschrift afgebeelde kooien gemaakt heeft. Ook de latere versie was van zijn hand. Hij kon alles maken en was een meester in het vertellen van mooie verhalen.

Minet de Wied en Inge Wolterink, paranimfen hors categorie. Vele jaren moesten jullie wachten tot jullie eindelijk aan de slag konden, maar toen het eenmaal zover was hebben jullie dat met grote energie gedaan. Minet, je bent een veel betere wetenschapper dan ik en ik hoop dat we onze vele discussies ooit nog een keer in een gezamenlijk artikel tot uiting mogen brengen. Inge, ik heb thuis mappen vol met werk wat door jou of onder jouw leiding gedaan is. Het was geweldig! Daarnaast was je op het RMI voor mij een bron van informatie en gezelligheid en gedurende al die jaren van spaarzame bezoeken een veilige haven.

Familie en vrienden, ik heb mijn best gedaan niemand onder mijn promotie te laten lijden en dat principe heeft ook voor een zekere vertraging gezorgd. Ik dank jullie voor de vaak voorkomende gelegenheid om het over iets anders te hebben dan over ratten.

Pa en ma, jullie bijdrage aan dit proefschrift is het grootst geweest. Als ik jullie liefde, hersens en nieuwsgierigheid niet mee had gekregen was ik er nooit aan begonnen en had ik het al helemaal niet afgemaakt.

Lieve Olof, Rosa en Marlou. Ik heb mij de afgelopen jaren nogal eens teruggetrokken omdat ik voor jullie een boekje aan het schrijven was. Dat boekje is nu gelukkig af. Ook was ik nogal eens in gedachten aan het piekeren en peinzen, dat was maar zelden vanwege dit boekje. Als jullie later groot zijn mogen jullie dus best wetenschapper worden.

Dankwoord

Lieve Marga,
Jij bent zonder meer degene die in de afgelopen 15 jaar dit proefschrift het meest heeft gefaciliteerd. Je hebt me altijd de ruimte gegund voor deze uit de hand gelopen hobby, de eerste anderhalf jaar heb je de kost verdiend en je hebt in de laatste jaren vaak de aandacht aan de kinderen gegeven die ik om wetenschappelijke redenen niet kon missen. En, hoewel je als geen ander het proces van dichtbij kon volgen, bleef je vertrouwen houden dat het ooit af zou komen. Ik houd van je en we gaan een rustige oude dag tegemoet!

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