From the Department of Medical Epidemiology and Biostatistics
Karolinska Institutet, Stockholm, Sweden

GENETIC AND HORMONAL INFLUENCES ON AFFILIATIVE BEHAVIOR IN HUMANS

Hasse Walum

Karolinska Institutet

Stockholm 2012

Published by Karolinska Institutet. Printed by Larserics Digital Print AB.

ISBN 978-91-7457-776-1

ABSTRACT

Affiliative bonds between romantic partners are widespread pan-culturally and an important part of human nature and society. Still, knowledge about the biological correlates of human pair bonds is sparse. Studies in rodents, voles in particular, have shown the neural circuits involving vasopressin and oxytocin, in males and females respectively, to be very important in the formation and regulation of pair-bonding behaviour. Further, both neurobiological and evolutionary studies have shown mate guarding behaviour to be closely linked to pair bonding.

In this thesis data from several twin samples from Sweden as well as from a randomized experimental study was used to investigate genetic and hormonal influences on pair-bonding behaviour and jealousy. Primarily, factors related to vasopressin and oxytocin were investigated.

Variation in the vasopressin receptor 1a gene and the oxytocin receptor gene was shown to be associated with pair-bonding behaviour in men and women, respectively. Further, intranasally administered oxytocin was shown to influence pair-bonding related behaviour in women. Finally, in accordance with evolutionary theory, men and women scored differently on quantitatively assessed jealousy. This difference was not detected on a genetic level. Genetic modeling analyses did however reveal that about 30% of the variance in jealousy is explained by genetic factors in both men and women.

Taken together, these results suggest that the well characterized effect of vasopressin and oxytocin on pair bonding in voles may be of relevance for humans. Also, the results from the quantitative genetics analyses, showing that genes are of importance for jealousy, make it interesting to hypothesize that the genes associated with pair bonding could influence jealousy as well.

LIST OF PUBLICATIONS

I. Hasse Walum, Lars Westberg, Susanne Henningsson, Jenae M. Neiderhiser, David Reiss, Wilmar Igl, Jody M. Ganiban, Erica L. Spotts, Nancy L. Pedersen, Elias Eriksson & Paul Lichtenstein. (2008). Genetic variation in the vasopressin receptor 1a gene (*AVPR1A*) associates with pair-bonding behavior in humans. Proc Natl Acad Sci U S A, 2008. **105**(37): p. 14153-6.

II. Hasse Walum, Paul Lichtenstein, Jenae M. Neiderhiser, David Reiss, Jody M. Ganiban, Erica L. Spotts, Nancy L. Pedersen, Henrik Anckarsäter, Henrik Larsson & Lars Westberg. (2012). Variation in the oxytocin receptor gene (*OXTR*) is associated with pair-bonding and social behavior. Biological psychiatry, 2012. **71**(5): p. 419-26.

III. Hasse Walum, Siri Leknes, Martin Larsson, Paul Lichtenstein, Bruno Laeng & Lars Westberg. Intranasally administered oxytocin increases pair-bonding related behavior in women. (Manuscript)

IV. Hasse Walum, Henrik Larsson, Lars Westberg, Paul Lichtenstein, Patrik K.E. Magnusson. The heritability of Jealousy. (Manuscript)

CONTENTS

LIST OF ABBREVIATIONS

AVP	Vasopressin
OT	Oxytocin
V1aR	Vasopressin receptor 1a
avpr1a	Rodent vasopressin receptor 1a gene
AVPR1A	Human vasopressin receptor 1a gene
AVPR1a	Primate vasopressin receptor 1a gene
OTR	Oxytocin receptor
OXTR	Oxytocin receptor gene
NAcc	Nucleus accumbens
TOSS	Twin and Offspring Study in Sweden
TCHAD	Twin Study of Child and Adolescent Development
CATSS	Child and Adolescent Twin Study in Sweden
SALTY	The Screening Across the Lifespan of Twins Younger sample
PBS	Partner Bonding Scale
RQS	The Relationship Quality Survey
CBCL	Child Behavior Checklist
A-TAC	Autism – Tics, ADHD, and other Comorbidities inventory
ASD	Autism Spectrum Disorder
MZ	Monozygotic
DZ	Dizygotic

1 BACKGROUND

Affiliative behavior constitutes a broad range of forms, including parent-offspring bonding, gregariousness among kin and friendly alliances between unrelated individuals. However, social interactions among adults are closely tied to reproductive opportunities [1]. Monogamy refers to the practice or condition of having a single partner during a period of time, a term applicable to social behaviors among certain species. Biologists commonly separate between different types of monogamy. Social monogamy infer dyadic coalitions between sexual partners based on selective social attachments (also known as pair-bonds), often characterized by partner preference, biparental care and intrasexual aggression. Sexual monogamy refers to sexual exclusiveness between partners. While social monogamy is relatively common in the animal kingdom, especially among birds, strict sexual monogamy is rare as individuals from socially monogamous species often engage in extra-pair copulations. It is debatable whether humans are monogamous by any definition. However, lasting bonds between sexual partners are widespread throughout nearly all modern human societies and although extra-pair copulations are common no matter the fact that the social organization is based on marriage [2], many individuals practice sexual monogamy. Monogamous behavior among humans is suggested to be shaped by evolution to reinforce bi-parental care in order to promote survival and prosperity of offspring [3, 4] or alternatively, as a consequence of male mating competition [5, 6]. Still, there are apparently large amounts of variation in monogamous behavior among our species and quantitative genetics studies of human mating behavior have found evidence of genetic influences on variation in reproductive behavior and sexual monogamy [7-10] as well as in more pair-bonding related outcomes [11-14].

Characterizing the biology of human affliative behaviour is challenging task. However, by implementing information gained from animal studies that have generated insights into the neural and genetic regulation of social behaviour, candidates for neural circuits possibly involved in human affiliative behavior can be studied. The neuropeptides vasopressin (AVP) and oxytocin (OT) have been proven to play central roles in the neurobiology of affiliative behaviour and social bonding in animals. Both are nonapeptides and are mainly synthesized by neurons of the paraventricular nucleus of hypothalamus. They differ structurally only at two amino acids and share the same evolutionary origin [15]. OT, best known for its role in peripheral circulation, particularly in contraction of the uterus during labour and ejection of milk during lactation, is implicated in a wide range of social behaviours including social motivation and approach behaviour when centrally released [16]. AVP, known for its actions as an anti-diuretic hormone, regulates several male-typical behaviours, including intrasexual aggression and paternal care [17-19]. Both OT and AVP are important for the formation and expression of social memory including parent-offspring recognition and mate recognition [20]. Probably the most striking effect of both these neuropeptides is however their central roles in the formation and regulation of pair bonding in voles [21].

1.1 VASOPRESSIN AND PAIR-BONDING BEHAVIOR

In contrast to other animals often used in the laboratory, prairie voles (*Microtus ochrogaster)* are very social and often form long-term, socially monogamous relationships with their partner. Both prairie vole parents contribute nearly equally to rearing the offspring. In contrast, the related vole species meadow vole (*Microtus pennsylvanicus*) and the montane vole (*Microtus ochrogaster*) are less social, do not readily form social bonds, and males give little attention to offspring.

In laboratory experiments pair-bonding behavior in male prairie voles is facilitated by vasopressin and prevented by an vasopressin receptor 1a (V1aR) antagonist [22]. Furthermore, the neuroanatomical distribution of V1aR differs considerably between these vole species, especially in the ventral pallidum where prairie voles display higher V1aR density [23]. In similar, other pair-bonding species, i.e. common marmosets (*Callithrix jacchus*) and the California mouse (*Peromyscus californicus*), display higher levels of V1aR in the ventral pallidum compared to related non-monogamous species [24, 25]. Moreover, partner preference is enhanced in the non-monogamous meadow vole when, by using viral vector gene transfer, V1aR density in ventral pallidum is increased [26].

Studies in voles have also provided intriguing insights into the genetic mechanisms explaining species differences and individual variation in V1aR distribution as well as in pair-bonding behavior. The gene sequence encoding the V1aR (*avpr1a*) include a 428 base pair sequence found upstream of the gene in prairie voles, but not in montane or meadow voles. Further, it has been shown that when the *avpr1a* of the prairie vole, including the sequence in the 5´ region, is transgenically inserted into the brain of mice [27] individuals from this normally promiscuous species show partner preference behavior accompanied with a V1aR expression, similar to what is seen in prairie voles. Furthermore, the 5´ flanking sequence of prairie vole *avpr1a* is polymorphic and variation in this gene affect V1aR expression and also alters intraspecific variation in pair-bonding behavior [28].

1.2 OXYTOCIN AND PAIR-BONDING BEHAVIOR

In similar to the actions of vasopressin on pair-bonding behavior in male voles, OT has a central role in the formation and regulation of pair-bonding behavior in female voles [21]. In prairie voles central infusions of OT facilitate [29] and a selective oxytocin receptor (OTR) antagonist inhibits [30] mating-induced partner preference formation in female individuals. Moreover, there are notable differences in OTR distribution patterns prairie voles compared to montane voles, mainly in the brain region nucleus accumbens (NAcc) [31], and an OTR antagonist applied directly to this region blocks partner preference formation in prairie voles [32]. Also, overexpression of the OTR in NAcc, using viral vectors, accelerates partner preference formation in female prairie voles [33]. The molecular mechanism behind the differences in OTR expression is not yet described. However, differences in elements in the oxytocin receptor gene (*OXTR*), potentially reflecting variation in gene expression, have been found between prairie and montane voles [34]. Similar to what has been shown in prairie voles the pair bonding common marmoset has high density of OTR in NAcc [24], and manipulations

of OT activity alter pair-bonding behavior in the closely related black-tufted marmoset (*Callithrix penicillata*) [35].

1.3 NEUROPEPTIDES IN HUMANS

Studies of the effects of AVP and OT on human pair-bonding behavior are rare. However, several studies have shown associations between variation in genes related to AVP and OT function and different aspects of social behavior. After the discovery that neuropeptides can be delivered to the brain in humans using nasal spray [36] a considerable number of experimental pharmacological studies have also been conducted and many findings of positive effects of intranasally administered neuropeptides on a wide spectrum of social behaviors have been revealed.

Most of the investigations of genetic variation related to AVP and OT have focused on genes coding for receptors for these peptides. The human vasopressin receptor 1a gene (*AVPR1A*) is situated on chromosome 12q14–15 [37], and several genetic variants situated in or close to the gene have been reported. Most attention has been given to three polymorphic repetitive sequences in upstream region of the gene: a $(GT)_{25}$ dinucleotide repeat, a complex $(CT)_4$-TT-$(CT)_8$-$(GT)_{24}$ motif (RS3) and a $(GATA)_{14}$ tetranucleotide repeat (RS1) [38]. Variation in both the RS1 and RS3 repeat has been linked to autism [39-43], age at first sexual intercourse [44], promoter activity in a human neuroblastoma cell line [43] and activation of the amygdala [45]. The RS3 repeat has also been associated with altruistic behavior [46, 47], prepulse-inhibition [48], impulsive aggression [49] and mRNA in postmortem hippocampus [46].

Studies of variation in the human oxytocin receptor gene (*OXTR*), localized on chromosome 3p25, have primarily investigated if single nucleotide polymorphisms (SNPs) in this gene are associated with different measures of social behavior. Similar to *AVPR1A*, variation in *OXTR* has been shown to be linked to autism [50-59]. Further, positive associations between SNPs in this gene and other measures of social interplay have been shown, including empathy [60] attachment style in patients with depression [61], social cognition in ADHD [62], emotional support seeking [63], prosocial temperament [64], maternal sensitivity [65] and prosocial decision making [66]. There is also some evidence suggesting that variation in *OXTR* is associated functioning [64] as well as the size of the amygdala [67, 68], a brain region known to be of importance for the regulation of social behaviors.

Intranasally administered AVP has been illustrated to alter the perception of social stimuli in a sex-dependent manner [69]. In men, intranasal application of vasopressin decreased the perception of friendliness in the faces of unfamiliar men and stimulated agonistic facial motor patterns. In women, vasopressin increased the perception of friendliness in the faces of unfamiliar women and stimulated affiliative facial motor patterns. More recent studies have shown administration of AVP to affect recognition of social words [70], encoding of both happy and angry social information [71] and men's recognition of sexual cues [72]. Intranasal AVP has also been shown to have effects on social behavior in other primates. Interestingly, administration of AVP influences pair-bonding behavior in the monogamous coppery titi monkey (*Callicebus cupreus*) [73].

OT administered through the nasal cavity have been shown to affect social behavior in a variety of studies [74-81], recently reviewed by Bartz et al. [82]. Most of these studies have investigated phenotypes not related to reproductive behavior, with some exceptions. Unkelbach et al. have demonstrated that intranasal OT facilitates recognition of sex and relationship related words [83]. Further, Ditzen et al. have shown OT to influence communication and behavior in a conflict discussion between couples [84].

As in voles and other animals, AVP and OT are released in humans during sexual intercourse [85, 86]. Moreover, a recent study has shown that plasma levels of OT are significantly higher in individuals having a partner compared to singles [87]. This is similar to what has been shown in the monogamous primate cotton-top tamarins (*Saguinus oedipus*). In this species, affiliative behavior between mates explains a large proportion of variation in OT levels [88].

1.4 PAIR BONDING AND MATE GUARDING

A lot of attention has been given to hypotheses explaining the evolution of social monogamy by benefits in biparental care of offspring [89, 90], a theory, it seems, not so applicable to mammals in general [91] and primates in particular [92]. In an effort to widen the perspective of monogamy evolution Brotherton and Komers [93] have recently considered different origins of social monogamy in mammals. They identify, based on phylogenetic data, three possible evolutionary routes to social monogamy. By using the monogamous dwarf antelope Kirk's dik-dik (*Madoqua kirkii*) as a model organism they conclude that male mate guarding best explain the evolution of social monogamy. One observation made in the dik-dik, related to the potential cost of roving (a situation when one male overlaps several female territories and also with extensive intrasexual overlap between males), is that males of this species over-mark their partners signs of oestrus by scraping dirt on top of the female urine and dung deposits. This mate guarding tactic makes it unlikely that an alternative polygynous strategy would be effective since over-marking makes it difficult for males other than those who are pair-bonded to assess when females ovulate.

Human females are sexually receptive throughout their reproductive cycle. In contrast, most mammals are receptive only a few days in each menstrual cycle. Humans also lack external cues to the imminence of ovulation also in contrast to other primates including chimpanzees. This absence of ovulatory signs (commonly termed "concealed ovulation") has raised a lot of interest among evolutionary biologists, not the least how this trait associates with monogamous behavior. Sillén-Tullberg and Møller [94] have constructed a phylogenetic tree of changes in visual signs of ovulation and found that monogamy more often evolve in lineages that lack ovulatory signals. It is possible that the presence of concealed ovulation, originally evolved to induce confusion about paternity and therefore avoid infanticide [95], made it difficult for ancestral human males, as for male dik-dik, to withhold a polygynous mating strategy. Instead a resident, mate guarding tactic would have been more efficient, suggesting an evolutionary link between pair bonding and mate guarding. This association is in animals testable by studying the effect of experimental removal of a mate guarding male on the frequency of extra pair-copulations [96]. Clearly, unfaithfulness in humans is most likely to happen when the partner is absent.

Considering the evolutionary origin of the relationship between pair bonding and mate guarding, evolutionary psychologists have suggested that the human psychological expression of mate guarding (jealousy) differ between the sexes. The theory is based on the assumption that the costs and benefits of pair-bonded mating are sexually dimorphic. Ancestral men's uncertainty regarding paternity exerted selective pressures that increased men's jealousy in response to sexual infidelity, whereas ancestral women's challenge of ensuring paternal investment exerted selective pressures that increased women's jealousy in response to emotional infidelity [97].

Besides the suggested evolutionary connection between mate guarding and pair bonding there seem to be a neurobiological link between these two phenotypes. In addition to the partner preference inducing effects of AVP in male prairie voles, this peptide facilitates mate-guarding behavior and a V1aR antagonist blocks intrasexual aggression in male of this species [98]. Further, Aragona et al. have investigated the importance of dopamine receptor D1 and D2 activation in the NAcc for pair-bonding behavior. They show that activation of the D1 receptors prevent pair-bond formation, while activation of D2 receptors facilitate this behavior in male prairie voles [99]. Interestingly, they also show that mating induced selective aggression in male voles is explained by upregulation of D1 receptors in NAcc, as blockade of these receptors abolish mate-guarding behavior [100].

Taken together, there is an obvious biological relationship between mate guarding and social monogamy. Therefore it important to, when studying the biology of human pair-bonding behavior, include assessments of infidelity avoidance tactics, including jealousy.

1.5 SEX DIFFERENCES

An underlying theme of my doctoral studies has been the study of sex differences. Sexually dimorphic traits are a controversial topic in politics and social sciences. Nonetheless, the study of sex differences has a long tradition in biology. Sexual differences are widespread in the animal kingdom and Charles Darwin [101] drew attention to these differences and presented several explanations for their evolution. The most popular theory of the evolution of sex differences is Darwin's sexual selection hypothesis, proposing that sexual dimorphism evolves when traits that constitute an advantage on the mating market are selected for in one sex. Although sexual selection probably is a valid explanation to much of the differences between sexes observed in nature, this is probably not the sole explanation to sexual dimorphism [102]. Natural selection due to ecological differences between the sexes [103] or fecundity selection [104] are alternatives to the sexual selection hypothesis as a cause of sexual dimorphism. No matter what hypothesis is adopted, a constraint on the genetic level exists [105].

Males and females share almost all their genes, but sex specific factors can interact with genes resulting in different gene expression patterns between the sexes. Sex differences in the genetic regulation of a trait can be quantitatively assessed by measuring the genetic correlation between the sexes. Such a correlation indicates

that male and female traits do not evolve independently. Although theoretical work have shown that different selection regimes for sexual dimorphism are unequally limited by the level of genetic correlation between the sexes [106], the evolution of sex differences is hardly possible if this correlation is equal to 1.0.

The idea of the impact of genetic correlation between the sexes on sexual dimorphism also dates back to the time of Darwin, although he referred to the concept by “the law of equal transmission”. For example Darwin believed that human mental capacities evolved in men through sexual selection, and wrote in the *Descent of Man* [101] that “It is, indeed, fortunate that the law of the equal transmission of characters to both sexes has commonly prevailed throughout the whole class of mammals; otherwise it is probable that man would have become as superior in mental endowment to women, as the peacock is in ornamental plumage to the peahen.”

In human quantitative genetics studies the concept of genetic correlation between the sexes is often referred to as “qualitative sex differences in heritability”. The presence of such a sex difference indicates a genetic correlation between the sexes less than unity.

2 AIMS

The overall aim of my thesis has been to gain better understanding of the biological correlates of pair-bonding behavior in humans. More specifically, the following questions were addressed in each study:

Study I. Does variation in microsatellites situated in the 5' region of the vasopressin receptor 1a gene associate with human pair-bonding behavior?

Study II. Is single nucleotide variation in the oxytocin receptor gene associated with human pair-bonding behavior?

Study III. Can intranasally administered oxytocin influence pair-bonding related behavior in women?

Study IV. Can sex differences in quantitatively assessed jealousy be identified in a large genetically informative sample?

3 METHODS

3.1 SAMPLES

The data used for the studies in this thesis came from several Swedish twin samples as well as a sample of primarily college students from the University of Oslo. A description of the samples used and the measures used in each sample is given below.

3.1.1 The TOSS sample

3.1.1.1 Subjects

The TOSS sample includes 909 twin pairs who are mothers (559 pairs) or fathers (350 pairs) of at least one adolescent child (254 MZ and 285 DZ twin mother pairs and 128 MZ and 183 DZ twin father pairs), their long-term partner or spouse, and one adolescent child of the twin parent. The recruitment of this complex twin family sample was possible by using the Swedish Twin Registry. The TOSS consists of two cohorts collected approximately 3 years apart. The first cohort consisted of 326 pairs of twin mothers drawn from female-female twin pairs born between 1926 and 1966, and their spouses. This sample was extended by adding a second cohort of twin mother and twin father families. The second cohort was drawn from same-sex twin pairs born between 1944 and 1971. Each member of the twin pair was involved in a long-term relationship with a partner residing in the same home. For inclusion in the sample, each twin was also required to have an adolescent child, ranging in age from 11 to 22 years (mean child age=15.7 ± 2.4 years), the same sex as the co-twin's child (49% males) with no more than a four-year age difference between the cousins. Twin fathers were slightly older than twin mothers, with average ages of 47.0 (+ 4.7 years) and 43.6 (+ 4.6 years) for fathers and mothers, respectively. Although it was not a requirement for inclusion, 96% of the spouse/partners were biologically related to the adolescent. These inclusion criteria were necessary to ensure that the current living experiences of each of the twin parents were comparable to his or her cotwin and their family members. Participants were mostly middle class, and consistent with the population of Sweden, in principle 100% Caucasian. Because the original 326 pairs of twin mothers were recruited and assessed 3 years earlier than the 350 pairs of twin fathers and additional 233 pairs of twin mothers from cohort 2, cohort effects were systematically examined prior to combining them for analysis. For the vast majority of the measures examined in TOSS there were no significant and meaningful differences between the cohorts.

3.1.1.2 Collected phenotypic data

The focus of TOSS is on family relationships and adjustment of adult twin parents [12, 107, 108]. The measurement package reflects this focus by assessing five broad categories of constructs: parent and child relationships with current family, parent's social experiences, parent adjustment, child adjustment, and individual attributes of family members. Also, twin specific factors and demographics were assessed. In many cases the respondent reports about themselves and about other family members.

3.1.1.3 PBS

The partner bonding scale (PBS) was created in order to measure pair-bonding behavior using questionnaire material from human subjects [109]. In accordance with

the behavioral domains observed when studying pair-bonding among non-human primates [5], items were collected from the Dyadic Adjustment Scale (DAS) [110], a frequently used assessment of the quality of marital relationships and similar dyads, the Support Seeking and Giving (SSG) [111] assessment measuring subjects' engagement with other people, and the Marital Instability Scale (MIS) [112]. Out of a total of 49 items, 18 questions (7 DAS, 10 SSG and 1 MIS) were considered relevant measures of human pair-bonding. A factor analysis was performed and items with loadings less than 0.4 on the first principal component were excluded resulting in the final Partner Bonding Scale which were created as the sum of 13 items (7 DAS, 5 SSG and 1 MIS). The reliability for this scale as measured with Cronbach's alpha was 0.79.

3.1.2 The TCHAD Sample

3.1.2.1 Subjects

The Twin Study of Child and Adolescent Development [113] includes all twins born between May 1985 and December 1986 alive and living in Sweden in 1994. The twins and their parents have been contacted in four different waves; when the twins were 8-9, 13-14, 16-17 and 19-20 years old. In wave 1, the parent questionnaire had a response rate of 75 % (*n* = 1 103). In wave 2, 73 % of the parents (*n* = 1 063) responded to the questionnaire. In wave 3, the parent-questionnaire had a response rate of 76 % (*n* = 1 067). In wave 4, both parents were approached separately, giving 1158 responses from at least one of the parents (mothers only: *n*=1061, fathers only: *n*=795), while self-reports had a response rate of 59% (*n*=1705). Excluding those with unknown zygosity and/or no available data, this study included 518 male monozygotic (MZ) twins, 366 male dizygotic (DZ) twins, 548 female MZ twins, 398 female DZ twins, and 440 opposite-sex DZ twins.

3.1.2.2 Collected phenotypic data

The TCHAD study is a longitudinal study of how genes and environments contribute to the development of health and behavior from childhood to adulthood. Major domains measured (at the different waves) are sociodemographic factors, physical health, behavior, personality, externalizing behavior and symptoms, internalizing behavior and symptoms, life events, and relationships [113].

3.1.2.3 RQS

The Relationship Quality Survey (RQS) [114], modified from the Network of Relationships Inventory [115], was used to assess positive and negative dimensions of the adolescents' reports of interactions with boyfriend/girlfriend in the TCHAD sample. For each item, ratings were made on a 5-point Likert scale (1 = not at all, 5 = extremely). The positive scale of this measure is comprised of eight items that assess warmth and support, including such items as, "How affectionate you are with this person?" and "How much does this person understand you?" The 4-item negative scale assesses conflict and negativity, including such items as "How much does this person criticize you?" and "How much does this person get into disagreements or fights with you?"

3.1.2.4 CBCL

In the TCHAD sample child behavior was assessed with the Child Behavior Checklist (CBCL) [116]. Sum scores were calculated for each of the eight syndrome subscales of the CBCL 1991 version (withdrawn, somatic complaints anxious/depressed, social

problems, thought problems, attention problems, delinquent behavior and aggressive behavior). Parents to twins were instructed to rate behavior "now" or "within the past 6 months" using a 3-point scale (0 = not true; 2 = very true).

3.1.3 The CATSS sample

3.1.3.1 Subjects

Parents of all 21,790 Swedish 9- (i.e. born July 1995-) and 12-year-old (i.e., born July 1992-June 1995) twins were identified through the Swedish Twin Registry and contacted for interviews over the phone as part of the Child and Adolescent Twin Study in Sweden (CATSS) [117]. The reason for choosing this age group was that most of the major child psychiatric problem constellations have been established by then, whereas the complex biopsychosocial problems associated with puberty have not yet emerged. Interviewers from a professional company, "Intervjubolaget", carried out the interviews after a brief introduction in child and adolescent psychiatry and twin research. The study started in July 2004 and is ongoing. As of November 2009, 80% of the parents of the cohorts born before May 2000 have responded. The mother was interviewed in 88% and the father in 12% of these cases; in 30 cases (0.4%) another member of the family was interviewed. The total sample consisted of 12 446 individuals and about 30% were monozygotic twins. In my second study genetic material from the first DNA collection from CATSS was used, including information from 1771 individuals.

3.1.3.2 Collected phenotypic data

The major aim of CATSS is to establish a longitudinal, nation-wide database on somatic and mental health problems in twins, with a focus on neurodevelopmental problems. Major domains measured in the data collection at age 9/12 are demographics, birth information, weight and length, physical health, prescribed medications, symptoms of somatic problems, psychiatric symptoms and problematic personality traits [117].

3.1.3.3 A-TAC

Child neuropsychiatric problems were identified using the Autism – Tics, ADHD, and other Comorbidities inventory (A-TAC); a comprehensive parent interview focusing on child autism spectrum disorders (ASD) and associated conditions validated for administration by lay persons over the phone [118]. The instrument includes questions to cover 96 specific child psychiatric problems, organized in modules theoretically defined in accordance with established diagnostic categories. Each module is assessed separately without diagnostic hierarchies. Questions are answered in a life-time perspective and in relation to age peers. Three response categories are used: "no" (0), "yes, to some extent" (0.5), and "yes" (1.0) [118, 119]. Test-retest reliabilities and inter-rater reliability for autism spectrum disorders, ADHD, developmental coordination disorder, tic disorder, and learning disorders were good to excellent (intra-class correlations at 0.87 or above). To improve possibilities to assign research proxies for clinical diagnoses, the scales were modified and a new validation with interviews with parents awaiting clinical neuropsychiatric investigations [119]. The autism spectrum is assessed by 17 gate items (6 for language, 6 for social interaction and 5 for flexibility) with optimal screening properties and 28 items assessing a wider range of autistic-like problems and peculiarities. The combined sum total ASD score (ranging from 0-45) has excellent psychometric properties to identify subsequent clinical ASD diagnoses assigned independently from

A-TAC interviews (area under Receiver Operating Characteristics (ROC) curve =0.96, [119]). In my second study we used A-TAC scores comprising all autism related A-TAC items for the three domains separately as well as the A-TAC total autism spectrum score.

3.1.4 The SALTY sample

3.1.4.1 Subjects

The Screening Across the Lifespan of Twins Younger (SALTY) study is a collaborative effort between researchers in epidemiology, medicine, economics, and political science initiated in 2007. In the beginning of 2009 SALTY was sent out to 24,914 Swedish twins born between 1943 and 1958 and the final reminders were sent out in the spring of 2010. The survey generated a total of 11,663 responses. Out of these, 11,341 (97.2%) respondents gave informed consent to have their responses stored and analyzed. Median birth-year is 1950. Sex-distribution is 54.3% females. Zygosity was resolved either by questionnaire items with high reliability or, when available, by analysis of biosamples. In total the sample is comprised of 1141 MZ pairs, 1222 same-sex DZ pairs, 1104 opposite sex DZ pairs and 9 showed an ambiguous zygosity. Remaining responses were from individuals whose twin siblings failed to respond.

3.1.4.2 Collected phenotypic data

The questionnaire data from the SALTY study covers a wide spectrum of assessment of behavior and somatic disorders, including coronary disease, economic behavior, psychiatric epidemiology and behavior in romantic relationships such as jealousy**.**

3.1.4.3 Jealousy measures

In the SALTY sample Swedish translations of two hypothetical infidelity questions suggested by Harris [120] were used:

Sexual jealousy: "You suspect that while your boyfriend/girlfriend was on vacation s/he had a one night stand. You realize that even if s/he did have sex with this other person, they will probably never see each other again. How upset do you think you would feel if this happened?"

Emotional jealousy: "You suspect that while your boyfriend/girlfriend was on a trip s/he fell in love with someone else. You realize that even if s/he did develop these feelings, s/he will probably never see this other person again. How upset do you think you would feel if this happened?"

Both were answered on a 10-point scale: 1= not at all, 10= extremely.

3.1.5 The Oslo sample

3.1.5.1 Subjects

For study III, participants were recruited mainly through adverts on notice boards at Oslo University. In the recruitment phase, participants were told that they would partake in an experiment studying the effect of oxytocin on social memory. During the data collection, two subjects were tested in each session; one man and one woman. Although both men and women were given treatment, for the purpose of the study included in this thesis men were regarded as stimulus individuals (partners) for the female participants. A total of 49 women (mean age 22.5, *SD*=3.88) participated in the

study. Out of these 23 (47%) women were single and 26 (53%) were non-single (married or in a romantic relationship).

3.1.5.2 Collected phenotypic data

A number of phenotypic assessments were used in the Oslo sample, inspired by research on attraction [121], experimental economics [122] and ostracism research [123].

3.1.5.3 Explicit judgment

Participants were shown a number of pictures of persons, randomly presented on a computer screen to them. Each picture in the test was presented three times and during each presentation one of three different statements was shown; "I would like to get to know this person better", "I think I would like this person if I got to know him/her", and "I think this person is attractive". The participants indicated, on a 7-point scale, how much they agreed with the statement. The mean value of the three statements was calculated, for judgments of the experiment partner and unknown strangers separately.

3.1.5.4 Dictator game

The dictator game is a game in experimental economics designed to measure variation in altruistic behavior [122]. We used an altered version of the original game where participants were given 200 Norwegian crowns (NOK) to divide between (a) herself, (b) the experiment partner, and (c) a stranger (one of the participants that would partake in the very next experiment session).

3.1.5.5 Cyberball game

The Cyberball game [123] is a computerized ball-throwing game used for research on ostracism and social rejection. We developed a version of the original game to fit the purpose of our study. The animations and general design from the original program was kept, but some additional features were added. In the description of the game, the participants were told that they would play the Cyberball game together with the experiment partner and a stranger of the same sex as the partner. The computer program displayed two pictures representing these two players, but the game was in fact arranged and no other human players besides the participant were included. When receiving the ball, the participant could click on one of the two pictures to pass the ball to that player. The whole game lasted for four minutes. During the first two minutes of the game the likelihood that the partner or stranger would throw the ball to the participant was set to 0.5. During the last two minutes of the game the participant was ostracized by the partner; the program was set to make the partner stop throwing the ball to the participant. The number of throws that the participants threw to the partner and the stranger before and after ostracism was recorded.

3.1.5.6 Cyberball questionnaire

We used questionnaire items similar to those described in Zadro et al. [124] to assess the participants response to playing the Cyberball game. In the work by Zadro et al. items were separated by those assessing mood and those assessing needs, and needs items were divided into four factors. Our data, however, did not give empirical support for grouping items the same way as in Zadro et al. The scree-plot from a factor analysis of both mood and needs items showed that the four first factors

accounted for a substantial proportion of the variance (63%) while each subsequent factor accounted for a small amount of additional variance. The first factor comprised items regarding experiences of alienation when playing the cyberball game. Next, the second factor included items regarding positive feelings about playing the cyberball game. The third factor tapped into tension/jealousy- reaction during the game. Finally, the fourth factor included items about feelings of control over the cyberball game. Items loading on each factor were reverse scored where necessary and summarized.

3.2 EXPERIMENTAL PROCEDURE

In study III two separate rooms were used for the experiment; room 1 and room 2. The female participant (A) was first brought to room 1, where she got to read through and sign an informed consent document. The same procedure was done for the male participant (B) in room 2. The next step was individual photography of each participant. When both participants had been photographed, the oxytocin/placebo administration was initiated. Both participants were randomly assigned to the oxytocin or placebo group. Five shots of the nasal spray were administered to each nostril in an interchangeable pattern resulting in a total amount of 40IU OT in the treatment group. After the administration, the participant was instructed to wait in their rooms for 30 minutes; this to ensure that oxytocin had passed over the blood-brain barrier before the rest of the experiment continued. Now a picture exposure session took place. Then participant A was led to room 2, where the participants met each other for the first time. Now a brief social interaction session was initiated; the participants were instructed to play a sketch and mime game together. This game continued for 10 minutes, and was directly followed by a proximity test. When this test was completed, the participants did not meet each other anymore for the rest of the experiment. Now participant B was led to room 1 where he performed a reaction time test. Participant A stayed in room 2 and performed an explicit judgment test and dictator game. The order of the judgment test and the dictator game was reversed after half of the test sessions to counter balance a possible order effect between the two. When both participants were done with their respective tests and games, they switched rooms (participant B was temporarily placed in an empty room while participant A was transferred to room 1, this so they would not met again). Participant A then performed the reaction time test and participant B performed the judgment test and the dictator game. When each participant had completed their individual tests and games they, in their respective rooms, played the cyberball game and filled out the cyberball game questionnaire and a general questionnaire.

3.3 GENOTYPING

Two different kinds of genetic markers were investigated in studies included in my thesis; repeat polymorphisms and single nucleotide polymorphisms (SNPs).

The GT_{25} repeat polymorphism in *AVPR1A* was amplified with primers 5'-TGTCAGACAAAACGCTGTTC-3' (forward) and 5' TGTGGCTTTAAAAGTTATCCAG-3' (reverse), the RS3 repeat polymorphism was amplified with primers 5'-TCCTGTAGAGATGTAAGTGC-3' (forward) and 5'-gtttcttTCTGGAAGAGACTTAGATGG-3' (reverse) [39, 125, 126] and the RS1 repeat polymorphism was amplified with primers 5'-AGGGACTGGTTCTACAATCTGC-3' (forward) and 5'-ACCTCTCAAGTTATGTTGGTGG-3'

(reverse) [39, 125]. The fluorescently labeled DNA fragments were analyzed by size with automated capillary electrophoresis using an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems).

Genotyping of SNPs rs75775, rs1488467, rs4564970, rs53576, rs237897, rs237887, rs11720238 in *OXTR* was performed by KBioscience (http://www.kbioscience.co.uk) using the KASPar chemistry, which is a competitive allele specific PCR SNP genotyping system using FRET quencher cassette oligos (http://www.kbioscience.co.uk/genotyping/genotyping-chemistry.htm). The remaining five SNPs rs4686302, rs2254298, rs2268493, rs1042778 and rs7632287 in *OXTR* were genotyped using commercially available 5' nuclease (TaqMan) assays on an ABI Prism 7900HT instrument (Applied Biosystems, Foster City, CA, USA).

3.4 STATISTICAL ANALYSES

All association analyses in the twin materials were done using Generalized Linear Mixed Effects Models (GLMM) in the PROC GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC). This procedure enables adjustment for the dependent nature of twin observations. Treatment effects in Study III were statistically tested using two-tailed *t*-tests (PROC TTEST, SAS Institute, Inc., Cary, NC).

3.4.1 Twin modeling

In genetically related subjects such as twins it is possible to investigate to what extent individual differences (observed variation in a population) are explained by genetic and environmental factors. MZ twin pairs share all of their genes and DZ twin pairs share, on average, 50% of their segregating genes. Estimations of genetic contribution to a continuous trait can be obtained by comparing similarities in scores using intra-class correlation coefficients for MZ and DZ twin pairs. Under the assumption that family environments are equally similar for MZ and DZ twins, higher correlation in MZ compared with DZ twins indicates a genetic effect. DZ correlations higher than half the MZ correlations indicate shared environmental, whereas DZ correlations lower than half the MZ correlations suggest non-additive genetic effects. If the differences in correlation coefficients between MZ and DZ twins are of unequal magnitude in men and women this indicates quantitative sex differences in heritability. Qualitative sex differences (different genes influence a trait in men and women) are indicated when the intra-class correlation for same sex DZ twins differs from that of opposite sex pairs. Cross-twin cross-trait correlations (comparisons between trait 1 in twin A and trait 2 in twin B) can be used to assess the amount of genetic correlation between traits.

Quantitative genetic model fitting can estimate the magnitude of genetic and environmental influence for a trait. Model fitting is based on comparison of the covariance between MZ and DZ twins [127] and allows partitioning of the observed phenotypic variance into additive genetic factors (A) reflecting additive effects of different alleles, non-additive genetic factors (D) reflecting interaction effects between alleles at the same or different loci, environmental effects shared by both twins (C), and environmental effects unique to each twin (E). The effect of C and D in the classical twin design is confounded because the effects of C decrease the difference

between MZ and DZ twin similarity, while the effect of D increases differences in it. Thus, C and D cannot be estimated simultaneously in the classical twin model. The proportion of variation in a phenotype that is explained by additive genetic influences is referred to as narrow heritability. Broad heritability, which is the focus of this study, includes additive and nonadditive effects (ie., A + D). Phenotypically correlated traits can be analyzed with multivariate genetic modeling in order to assess the degree of the genetic overlap between the traits. The multivariate model estimates the genetic and environmental correlations, which varies from -1.0 to +1.0 and indicates the extent to which genetic and environmental influences in one phenotype overlap with those of another phenotype.

All genetic modeling was carried out with Mx software [128] (http://www.vcu.edu/mx).

4 RESULTS

4.1 STUDY I

Inspired by the investigations of *AVPR1A* and V1aR in relation to pair-bond formation in other species we investigated whether the three repeat polymorphisms located upstream of *AVPR1A* was associated with pair-bonding related traits in humans. We found, in the TOSS sample, that the RS3 repeat was significantly associated with scores on the partner bonding scale (PBS) in men ($F_{19,\ 157}$=2.48, p=0.001) but not in women, consistent with the fact that AVP and its actions through V1aR are most prominent in male voles. Further analyses showed that one specific allele, allele 334 of the RS3 repeat, was strongly associated with PBS scores in men ($F_{1,130}$=16.35, p<0.0001). This association also showed a dose dependent pattern; those carrying two 334 alleles had the lowest scores on the heterozygotes scored in between and non-carriers had the highest scores (Figure IA). We could also show that men carrying the two copies of the 334 allele were twice as likely to have experienced marital crisis with threat of divorce during the last year then other men ($F_{2,\ 143}$=5.00, p=0.008) (Figure IB). Finally, women married to men carrying this allele reported being less satisfied with their marital relationship than women married to men not carrying it.

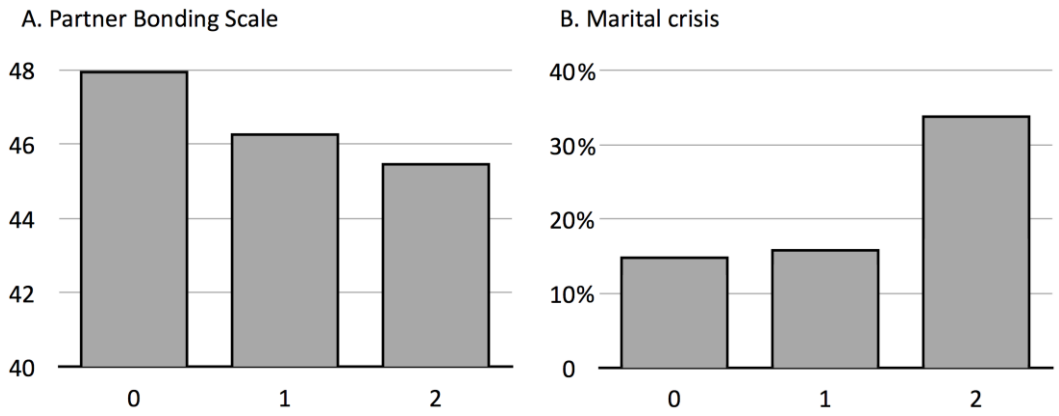

Figure I. Mean values for the Partner bonding scale (A) and percentage of individuals that have experienced marital crisis during the last year (B) for men divided by number of 334 alleles.

4.2 STUDY II

In analogy with our findings of an association between *AVPR1A* polymorphisms and pair-bonding behavior in men, we studied if variation in *OXTR* is associated with pair-bonding behavior in women. Twelve SNPs in the *OXTR* were investigated in the same sample (TOSS) as was used in study I. In parallel, the same SNP set was also genotyped in a separate sample (TCHAD) comprising measures of pair-bonding behavior (RQS) in young men and women in current romantic relationships. Intriguingly, the same SNP, rs7632287, was associated with self-reported pair-bonding behavior in the TOSS sample ($F_{2,1093}$=3.88, p=0.02) as well as in in the younger women in the TCHAD sample ($F_{2,238}$=3.16, p=0.04). Women carrying A-alleles scored lower on the pair-bonding measures than other women in both samples (Figure II). No consistent associations were however seen between the pair-bonding measures and any of the other investigated SNPs.

The rs7632287 SNP, positively associated with pair-bonding behavior in women in the first step, was further studied in relation to marital crisis and measures of marital quality as perceived by spouses in the TOSS sample. We found that women carrying the A-allele more often had experienced marital crisis with threat of divorce during the last year than women not carrying this allele ($F_{1,1084}$=9.03, p=0.003). Eleven percent of women carrying no copy of the A-allele reported marital crisis, whereas 16% of the women carrying this allele reported marital crisis. Furthermore, marital quality, as assessed by the husbands, was associated with the rs7632287 genotype of their wives. Men married to women carrying the A-allele had lower scores on a scale measuring dyadic consensus than men married to women not carrying this allele ($F_{1,1147}$=5.64, p=0.02).

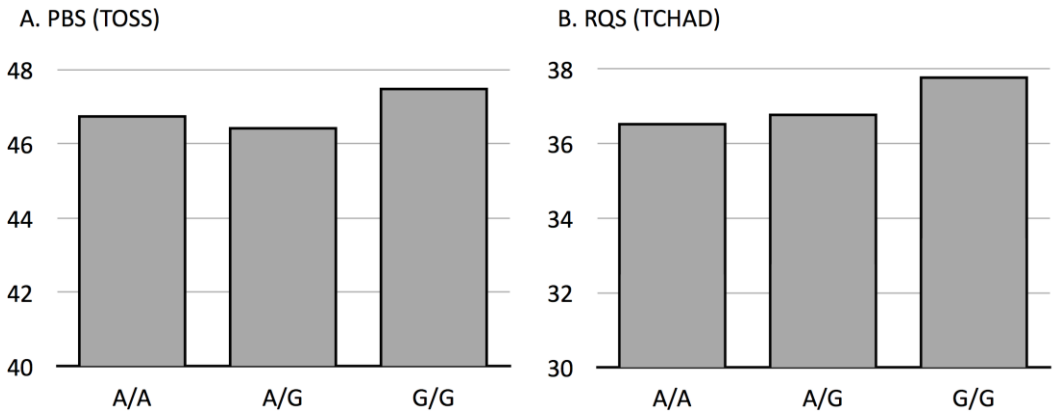

Figure II. Mean values for the Partner bonding scale (A) and the Relationship Quality Survey scale (B) for women divided by rs7632287 genotypes.

Next, the relation between behaviors in young girls and adult pair-bonding behavior was explored using the longitudinal design in TCHAD. To this end we used the Child Behavioral Checklist inventory (CBCL) reported by parents in the first wave of the TCHAD study (at age 8-9). The parent-reported CBCL social problems subscale (a scale assessing a variety of difficulties that children may display in their relationships with peers and adults), but no other CBCL subscale, was significantly associated with girls´ behavior in romantic relationships at ages nineteen to twenty years (r=-0.14, p=0.004). We then demonstrated that in girls the CBCL social problems scale was associated with the rs7632287 genotype ($F_{1,460}$=7.42, p=0.007). Consistent with the association between the rs7632287 SNP and pair-bonding behavior described previously, girls carrying the A-allele experienced more social problems than girls not carrying this allele. As the CBCL social problems scale previously has been shown to be associated with measures of the autism spectrum [129] we finally investigated to what extent the rs7632287 SNP was associated with symptoms of autism as assessed in 1771 children from the CATSS sample using parent interviews with the Autism-Tics, AD/HD, and other Co-morbidities inventory (A-TAC). In line with our hypothesis, in girls – but not in boys - the rs7632287 A-allele was associated with higher A-TAC total autism spectrum score ($F_{2,539}$=5.70, p=0.004) as well as with higher scores on the social interaction ($F_{2,494}$=6.94, p=0.001) and communication ($F_{2,557}$=3.36, p=0.04) domains in the autism spectrum.

4.3 STUDY III

In order to investigate the effect of intranasal OT on pair-bonding related behavior in women we first needed to identify what measures included in the study that captures this phenotype. To do so we performed a factor analysis. In this analysis five factors emerged explaining 68% of the variance. The first of these factors clearly related to women's affiliative response towards the male stimulus individual. Measures with high factor scores for this factor were; positive experience of playing the cyberball game, explicit judgments of experiment partner, money given to the experiment partner, number of throws directed towards the partner in the cyberball game before ostracism, and number of throws directed away from the partner after ostracism had occurred. These five items were standardized to a mean of 0 and a variance of 1 (PROC STDIZE, SAS Institute, Inc., Cary, NC) and then summarized. This scale was then used to assess the effect of OT on women's affiliation towards the experiment partner.

Consistent with animal data and in line with our hypothesis we saw that women given intranasal OT scored higher on the affiliation scale compared to women given placebo (t_{35}=2.16, p=0.04, d=0.72) (Figure III). Further, we found it reasonable to assume that the affiliation effect of OT could differ depending on relationship status, since individuals in a romantic relationship have been shown to have elevated oxytocin levels compared to those who are single [87]. As expected we saw a significant effect of OT on affiliation in single women (t_{16}=3.07, p=0.007) with even higher effect size (d=1.40), while the association did not reach significance in non-single women (t_{16}=0.84, p=0.41) (Figure III).

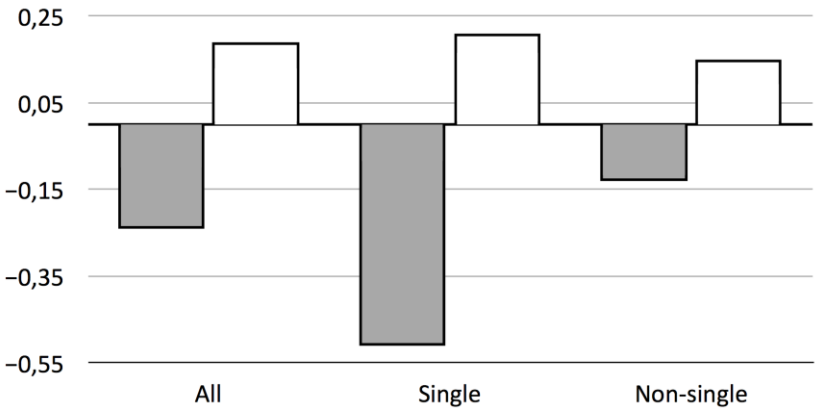

Figure III. Affiliation scale scores for women given placebo (gray bars) and OT (white bars) divided by relationship status.

4.4 STUDY IV

At first glance our data seemed to contradict the evolutionary theory of sex differences in jealousy. Women scored higher than men on both sexual (Women: 8.38, *SD* = 2.08; Men: 7.69, *SD* = 2.36) and emotional (Women: 7.15, *SD* = 2.50; Men: 6.16, *SD* = 2.65) jealousy. However, recent studies have made a strong case that the relationship between jealousy types is what should be studied in relation to sex [130, 131]. Consistent with this idea, the difference score (sexual jealousy scores subtracted by emotional jealousy scores) in men (1.53, *SD* = 2.04) was higher than in women (1.23, *SD* = 1.97), ($F_{1, 9329}$= 60.24, p < 0.0001), indicating that men, relative to women,

reported greater jealousy in response to sexual infidelity than in response to emotional infidelity.

We investigated sex differences on a genetic level by comparing twin correlations between males and females as well as between same sex and opposite sex DZ twins. No evidence of quantitative or qualitative sex differences in the heritability of jealousy was found as correlations were similar in both sexes and in same sex and opposite sex DZ twins (Figure IV). Further, cross-twin cross-trait correlations gave no evidence that the amount of genetic overlap between sexual and emotional jealousy differ in men and women.

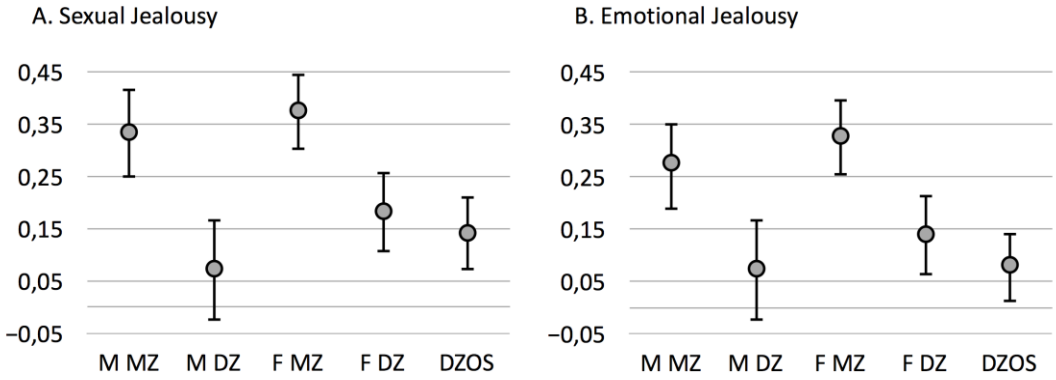

Figure IV. Intraclass correlations with confidence intervals by zygosity, sex and jealousy type.

Genetic modeling analyses were performed for men and women combined and included broad heritability (i.e., A + D) and non-shared environmental effects. Results from these analyses suggested that the broad heritability for sexual and emotional jealousy was 32% (95% confidence interval; 27%-37%) and 26% (95% confidence interval; 21%-31%) respectively. Almost identical variance components were found when accounting for mean differences of age and sex in the model. Multivariate analysis was also performed for men and women combined. This analysis revealed a genetic correlation between sexual and emotional jealousy of .83 (95% confidence interval; 0.77-0.89), indicating that the majority of genes impacting on sexual jealousy also affect emotional jealousy.

5 DISCUSSION

The neurobiological model for mating induced pair bonding in voles is based on the release of OT and AVP as a consequence of copulation. In prairie voles these neuropeptides bind to receptors influencing the neural circuitry involving dopamine in brain regions associated with reward and reinforcement. The effect of the activation of both neuropeptide and dopamine receptors in reward centers is a conditioned partner preference [132]. My interpretation of the results from the first three studies in this thesis is based on the possibility that the same neurobiological model to some extent also is applicable to human pair bonding (the reasonableness of the hypothesis that OT and AVP can be important regulators of pair-bonding behavior in distant taxa is discussed in section 5.1). Under this assumption, variation in the genes coding for V1aR and OTR (investigated in study I and study II respectively), that possibly reflect differences in gene expression, could affect the strength of the conditioned reinforcement induced by mating. Further, as the discussed model presumes that the mating-activated neuropeptidergic response translates sensory stimuli from the partner to a pronounced reinforcement, the effect of intranasal OT in my third study could substitute the release of this peptide during copulation and, when accompanied with social stimuli, result in bond. The sexually dimorphic effects found in these studies, that are consistent with animal research, could reflect sex differences in receptor or peptide expression, dependent on gonadal steroids [133].

Taken together, the results from the work presented in this thesis suggests that the well characterized actions of AVP and OT on vole pair bonding could be of importance also for human affiliative behavior. These findings need to be replicated in order to determine if they reflect true or spurious associations. Although very few other genetic and pharmacological studies in humans have focused on pair-bonding related behavior, there are several promising findings of associations between the *AVPR1A* and *OXTR* polymorphisms mentioned in this thesis, as well as intranasal AVP and OT, and different aspects of social behavior.

Regarding *AVPR1A*, some consistencies seem to appear when comparing studies investigating the RS3 repeat. Relatively shorter RS3 alleles, which have been associated with lower AVPR1A mRNA expression in the brain [46] and lower transcriptional activity *in vitro* [43], are associated with increased risk of autism [39, 40], less altruistic behavior [46], less prepulse inhibition [48], less amygdala activation to social recognition [45] and increased aggressive behavior [49]. This is in line with the vole studies showing associations between repeat length and V1aR density as well as intraspecific variation in social behavior. The 334 allele found to be associated with male pair-bonding behavior in my first study is one of the most common shorter alleles. Noteworthy is also that in several studies, this allele (which has been referred to as allele 334 and allele 327 in different studies dependent on which primers were used) seems to explain most of the association seen with the short-long dichotomization [39, 45, 47, 134]. One thing to keep in mind is however that the RS3 repeat is a complex polymorphism featuring two adjacent dinucleotide repeats that both are polymorphic [38]. This could implicate that genotype designation based on PCR product lengths do not capture the full extent of the variation at this locus. It is

possible that accurate genotyping using sequencing methods would increase the strength of the association or refine the genotypes implicated. Further molecular genetic studies are needed before we more precisely understand how the RS3 repeat polymorphism affects expression of the *AVPR1A*.

To date the rs7632287 SNP has been investigated in three studies of autism, including ours [54, 56, 135]. These three studies comprise the largest number of subjects of all the candidate gene studies of *OXTR* in relation to risk of autism conducted so far. Intriguingly, although they included most of the SNPs previously shown to be associated with autism, rs7632287 showed the strongest association in all three studies. The rs7632287 SNP is located in the 3' region of *OXTR* and in silico analysis suggests that this SNP afflicts transcription factor binding to the 3' end of the *OXTR* that may be important for gene expression levels [54]. However, further investigations are needed before we know if this SNP has any importance for the protein function per se or if it is in linkage disequilibrium with a functional variant.

My third study is the first attempt to try to investigate if knowledge about the role of OT in pair bonding in female voles can help us understand the biology behind the formation of human affiliative bonds. Therefore, there is not much further support from human studies that OT could induce social bonds in our species. However, the rapidly growing amount of studies showing pro social effects of intranasal OT favor the possibility that the results shown in our study could reflect a true effect. Further studies of neuropeptidergic effects on human pair bonding are warranted, including investigations of AVP that has been given less attention in human pharmacological studies than OT. To better understand the complexity of human romantic relationships it is also important to widen the phenotypic perspective. More pair-bonding related behaviors need to be investigated, including romantic jealousy.

5.1 VASOPRESSIN AND OXYTOCIN OVER EVOLUTIONARY TIME

Much of the work on the biological regulation of pair-bonding behavior is done in rodents. Are there reasons to believe that these findings are of importance for other evolutionarily distant species, such as humans? This question is of importance to evaluate the prior probability of the hypotheses presented in the studies in this thesis.

AVP and OT display a marked conservation in gene structure and expression [136, 137]. OT and AVP (when including the two AVP homologs Lysipressin and Phenypressin) are found in all mammals [138] and similar peptides are also found in invertebrates [139-141]. Among distant taxa, OT and AVP related peptides play a general role in the modulation of social and reproductive behaviors [138]. Further, within vertebrates, the OT and AVP peptide lineages often show sexually dimorphic expression and behavioral effects [142]. At the same time, as mentioned above, variation in genes coding for the specific receptors for OT and AVP seem to explain dissimilarities in social behavior, both within and between species.

The strong association between the *avpr1a* microsatellite and pair-bonding behavior in voles begs the question if this marker determines mating system in rodents, and if similar genetic regions have corresponding effects in other mammals. The first study

to call the relationship between the *avpr1a* microsatellite and mating system into question compared genetic information at this locus between different vole species [143]. They found, consistent with previous work, that the promiscuous montane and meadow voles lacked the avpr1a microsatellite but could also show that other vole species known to be promiscuous had long avpr1a microsatellites. Further, they showed, using cytochrome b sequences to construct a phylogenetic tree of vole species, that the presence of the long *avpr1a* microsatellite is the ancestral state suggesting that a deletion of region occurred in a common ancestor of montane and meadow voles. These findings contradict the hypothesis that the avrp1a microsatellite determines mating system in rodents, but as mentioned by Young and Hammock [144] they ”do not preclude the possibility that genetic variation in Avpr1a across species contributes to behavioral diversity, thereby influencing mating strategy”. There is compelling evidence that V1aR expression predicts mating behavior in voles [145, 146] and it is indeed possible that variation at different loci in avpr1a affects region specific expression that in turn associates with mating tactics [147]. In other words, other sources of variation in *avpr1a*, including more complex variants of the avpr1a microsatellite than captured by the length of repeats, might contribute to variation in mating behavior between species.

In 2005 Hammock and Young [148] suggested that repetitive non-coding DNA in the upstream region of *AVPR1a* could explain differences in social behavior among primates. This idea came from the discovery that both humans and bonobos (*Pan paniscus*) carry the complex RS3 repeat (investigated in study I) while this region is missing in chimpanzees (*Pan troglodytes*) and that this species difference could reflect differences in social behavior. However, further analyses of interspecies variation in *AVPR1a* among 12 species of Old World primates [149] show that there are no simple relationship between microsatellites in this gene and behavior. Similar as in voles [143] the presence or absence of repetitive polymorphisms in the region upstream of *AVPR1a* does not covary with the mating strategy males of different primate species adopt. Nonetheless, variation in the RS3 repeat has recently been shown to be associates with personality in chimpanzees [150].

The genetic regulation of differences in OTR expression among voles remain to be described and *OXTR* is not as thoroughly studied in different animals as *avpr1a.* In contrast, more direct effects of OT on affiliative bonding have been studied in a wide spectrum of species. As mentioned above, OT has been linked to pair-bonding in several species. Further, both partner preference and parental care is hypothesized to result from neuropeptide modulation of circuits regulating reward and reinforcement and those implicated in sensory information processing [151]. It has therefore been suggested that a neural system specialized to regulate maternal bonding could be evolutionarily transformed to modulate partner bonding as well. Indeed, OT has been shown to influence maternal behavior in rats [152], mice [153] and sheep [154]. Injections of an OTR antagonist into NAcc block spontaneous parental behavior in female voles [155]. Plasma levels of OT are also associated with parental behavior in humans [156]. Taken together, studies of parental and romantic attachment have provided substantial evidence that OT is an important regulator of affiliative bonding in mammals.

In summary, there is evidence from different parts of the animal kingdom that AVP and OT are both implicated in bonding behavior. Although the evidence in humans is not conclusive the work presented in this thesis gives an important contribution to the field by showing that the actions of neuropeptides could be similar in our species as in rodents and non-human primates. The relationship between genetic markers in neuropeptide related genes and bonding behavior in different species is not straight forward, and social monogamy can certainly have evolved through changes in neural systems other than those involving AVP and OT. This however does not contradict that variation in phenotypes related to monogamy can be explained by variation in AVP and OT related genes in specific species or that neuropeptides can facilitate bond formation in different lineages even though social monogamy has evolved independently in them.

5.2 EVOLUTION AND GENETIC VARIATION

A large part of the work in this thesis has focused on the effect of genetic variation on pair-bonding related traits. In study I and study II variation in candidate genes was investigated and shown to be associated with pair-bonding behavior. The main phenotypic measure used in these studies (PBS) was shown to be heritable. Also, about 30% of the variance in jealousy was shown to be explained by genetic factors in study IV.

Fisher's fundamental theorem of natural selection states that "The rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time" [157], or put in more modern terms: "The rate of increase in the mean fitness of any organism at any time ascribable to natural selection acting through changes in gene frequencies is exactly equal to its genetic variance in fitness at that time" [158]. In other words, according to this theorem, selection depletes additive genetic variation in fitness related traits. Why then, do we find genetic variation for pair-bonding related behavior in humans, traits indeed related to fitness and selected for over evolutionary time? The ideal conditions needed for selection to completely diminish genetic variation for these behaviors seem to rarely exist. There are three leading explanations from evolutionary genetic theory that could explain why:

1. Neutral evolution [159]: Variation in genes influencing a phenotype does not affect the fitness of individual organisms and is therefore not affected by selection. Variation can be neutral for different reasons; synonymous substitutions in DNA sequences, variation in pseudogenes and other non-transcribed DNA and neutral amino acid variation are examples of changes on the molecular level without evolutionary impact. Neutral variation introduced by mutation may, through random drift effects such as founder effects and genetic bottlenecks, become more common in a population and in rare cases even become fixed.

2. Mutation-selection balance [160]: Selection will act against deleterious alleles until they eventually are deleted from the population. Mutations with highly harmful effects will be deleted fast, while mutations with milder effects will be removed more slowly. Highly polygenic traits have a large

mutational target size, meaning that a large number of loci are affecting the trait. The likelihood of random mutation affecting a certain phenotype will increase with the mutational target size of the trait. Therefore, for some phenotypes mildly deleterious mutations can accumulate because new mutations are introduced at a higher pace (due to a large mutational target size) than the force of selection deleting this new variation.

3. Balancing selection: A number of selective processes actively maintain genetic variation in a population. These processes usually involve opposing forces in which selective advantages and disadvantages cancel each other out. Balancing selection processes include; heterozygote advantage (alleles disadvantageous in a homozygous state are retained because of selective advantages in a heterozygous form) [161], antagonistic pleiotropy (an allele increases fitness payoffs of one trait but reduces the payoffs for another trait) [162], sexual antagonism (an allele is favored in on sex and selected against in the other) [163], temporal and spatial variability (the fitness of different alleles varies over evolutionary time or space) [164] and negative frequency dependent selection (an allele is favored when rare and selected against when it is common) [165].

It is not within the scope of this thesis to give a detailed answer to the question which of the above theories best explain why selection have not depleted all genetic variation for monogamy related behavior in humans. I do however find it important to understand the mechanisms in play generating the variables one choose to study. After reviewing the literature it is my belief that both animal [166-168] and human [169, 170] studies of life history strategies, together with the neurobiological data mentioned in this thesis, suggest that balancing selection, especially temporal and spatial variability, fits well with the observed heritable variation in monogamous behavior. This would mean that genetic variation associated with pair-bonding related traits, observed in *AVPR1A* and *OXTR* in my first two studies and the quantitative genetic findings in my fourth study, remain in the population because of temporal or geographic fluctuations of selection pressures. I believe that these fluctuations have been at least to some extent consisted of differences in mate availability. Under circumstances when there is an abundance of potential mates, individuals that do not bond so strongly to their partner (men carrying the *AVPR1A* 334 allele and women carrying the A-allele of the rs7632287 SNP in *OXTR*) can gain fitness advantages in form of mating opportunities with others than their partner. But if the population density varies there will be situations when the number of available mates will be low and this will favor individuals that stay in close proximity of their partner and practice pronounced mate guarding (by displaying strong jealousy reactions). This variability can constitute a limit for directional selection to fixate alleles and genetic variation will remain in the population.

5.3 METHODOLOGICAL CONSIDERATIONS

5.3.1 Multiple testing and publication bias

If several statistical tests are performed simultaneously, multiple testing should be taken into account to properly control the false positive rate. This is of great importance in genetic association studies. Issues of multiple comparisons are in

genetics often addressed by using Bonferroni correction. With this approach the significance level is divided by the number of tests performed. When an extremely large amount of tests are performed, as in genome wide association studies, this approach can be problematic as large sample sizes are required in order to maintain statistical power. In the first two studies in my thesis a candidate gene approach was adopted and the choice of genes and markers was inspired by previous research in animals. Therefore, the number of markers investigated could be kept relatively low and the burden of having to control for many thousand tests (as in genome wide association studies) was avoided. Bonferroni correction was still used in these studies to reduce the risk of type I errors.

I have argued that the results I have presented are in line with animal data and that a growing amount of human studies is showing a quite consistent picture of how neuropeptides and genes related to them associates with social behavior. Undoubtedly, human studies within this field have been inspired by the elegant animal research showing remarkable pro-social effects of AVP and OT. Hypotheses about how these peptides affect humans are sprung from results from previous animal studies. This could potentially lead to publication bias as studies showing results in line with animal data will be attractive and could therefore have a higher probability of getting published. This should be kept in mind when reviewing the literature and conclusions about consistency should be drawn cautiously.

5.3.2 Issues with twin studies

There are several basic assumptions underlying the twin method including the equal environment assumption stating that similarity caused by environmental factors are roughly the same in both MZ and DZ twins, the assumption that mating occurs randomly in human populations and that twin studies are generalizable to a the population as a whole. Potential problems with these assumptions have been discussed at length [127]. Perhaps most important in the context of the twin study presented in this thesis is the question about generalizability. It is possible that having a twin sibling could influence an individual's jealousy reactions as an effect of extensive sharing during childhood. Although this might influence some types of jealousy and envy it is less likely that this is a large problem in the context of romantic jealousy. As mentioned above there are reasons to believe that mate guarding, and therefore jealousy, is induced by modulation of neural circuits with mating. If mating is necessary for experiences of romantic jealousy environmental factors during childhood might not be of large importance. Further, the scores for emotional and sexual jealousy in the SALTY sample are similarly distributed as in previous reports from singleton samples [130].

5.3.3 Measurement issues

The work in this thesis relies largely on self-report questionnaires for measures of pair-bonding related behavior. Self-reports can introduce bias, especially for questions regarding sensitive information such as behavior in romantic relationships, as people can be reluctant to answer truthfully. Efforts to address this have been done in the twin studies used. In the TOSS study for example, to ensure that subject's answers about family situations were not influenced by their partner, each participant were

accompanied by research personnel when filling out sensitive parts of the questionnaire. Although questionnaires can be an efficient way to collect behavioral data it is of importance to investigate if the associations described in this thesis can be replicated using measures more objective than self-reports. Research in psychology has a tradition of using experimental approaches when assessing human behavior. Recent studies have used interesting methods in order to measure individual differences regarding pair bonding [171] and jealousy [172]. These kinds of methods can in combination with genetics give further insights into the biology of pair-bonding related behavior in humans.

The phenotypic assessments used in study III are not designed to measure pair-bonding behavior per se. Laboratory studies of these kinds of behaviors in humans are very rare and our effort to conduct this original experiment came with the challenge of choosing an appropriate strategy for phenotypic assessment. The paradigms we chose to include are relevant and reliable and the factors revealed by the factor analysis are intuitively compelling. However, further studies of human bonding behavior should investigate a wider range of measures potentially reflecting variation in attachment and see how these variables depend on the relationship between subjects and stimulus individuals.

In study IV we used reactions to imagined infidelity as measures of jealousy. This could be problematic for several reasons. First, measuring jealousy reactions using hypothetical infidelity scenarios may be problematic since these measures do not necessarily correspond to reactions to real infidelity [173]. Further, using separate continuous measures for sexual and emotional jealousy does not implicate that these questions assess mutually exclusive entities [174]. Common stereotypes regarding how men and women behave in mating situations can implicate that study subjects perceive both infidelity types to be measures of roughly the same thing. If this is the case it is indeed possible that our results showing a large genetic overlap between sexual and emotional jealousy is an over estimation. In addition, our questionnaire items could capture several additional traits, such as reactions to cultural norms or relationship experiences, associated with the partner guarding adaption we wish to measure (this is probably applicable to the pair-bonding measures used in study I and study II as well). Such associations could affect the precision of the quantitative genetics analyses.

6 CONCLUSIONS

The motivation for the work I have done for this thesis has been to gain better understanding of the biology of human pair-bonding behavior. Research within this field can however also have more wide implications. There is a possible neurobiological relationship between variation in social behavior and disorders characterized by social impairment, such as autism. The results from my second study suggest that the OT system can be an example of such a link. While disruption in neuropeptide systems is not likely to explain a lot of the variance in autism, better understanding of the biological correlates of social behavior can indeed have future clinical implications for this disorder. Further, better understanding of what makes individuals bond with each other, or perhaps more importantly why bonds are broken, can be of importance for the society. The number of divorces is increasing and this trend has consequences for many parts of our social structure. There are reasons to believe that the massive cultural changes that has taken place during the last 10 000 years have occurred at a pace to fast to allow human brains to adapt. Our inherited mating strategies might not be geared for modern living. To fully understand the consequences of the potential mismatch between society today and our evolutionarily shaped behavior an integration of social science and biology is necessary.

In summary, we have used biological study designs to investigate human affiliative behavior. In study I we show that variation in *AVPR1A*, a gene shown to an important determinant of pair bonding in male voles, could be of relevance for this phenotype also in men. Study II and study III suggests that OT is linked to pair-bonding behavior in women, also consistent with animal data. This was demonstrated both by an association between *OXTR* and bonding behavior in a romantic relationship as well as the effect of intranasal OT on the formation of a pair-bonding related preference. If replicated in future studies these findings suggest that similar neural circuits may be implicated in pair bonding in humans as those characterized in voles. In study IV the phenotypic perspective is extended to include another important behavior related to pair-bonding; jealousy. The quantitative genetic analysis of this phenotype suggests that a substantial proportion of the variance is explained by genetic factors. By integrating psychology, evolutionary biology, social neuroscience, genetics and epidemiology I believe that this thesis have provided important new knowledge about social behavior in humans.

7 ACKNOWLEDGEMENTS

Paul Lichtenstein – My principal supervisor. I have been dissatisfied with what education has had to offer ever since first grade. Working with you and learning the scientific method through the conversations we have had over the years have changed that. Thank you for giving me the opportunity to become a researcher, for your never ending support and that you have always found the time to ask me how I feel.

Lars Westberg – My co-supervisor. One thing I will always remember from my time as a PhD-student is our conversations about research, both over the phone and at Göteborgs universitet. Your keen research interest is indeed inspiring. Thank you for your guidance, enthusiasm and for stimulating me to come up with new ideas.

Christina Hultman – My "external" mentor. You have many times made me feel that I am capable of performing beyond what I thought was possible. Thank you for the inspirational lunches at SMI.

Niklas Långström – You have been interested in and given smart comments to my most strange ideas. Thank you for listening.

Patrik Magnusson – I have spent a substantial amount of my hours at the department in your office talking about research and audio, how to combine them both and the worst of all evil things; jitter. I hope our joint quest to perfect things that are important will continue.

Henrik Larsson – Thank you for always taking time to talk to me and for giving me the help I needed when I really needed it.

Emma Frans – Everyone should have a best friend at work. I am glad that you have been mine. Thank you for pointing me in the right direction when I have been a bit lost.

Thomas Frisell and **Therese Ljung** – You, I and Emma have in ways done this journey together. Thank you for contributing to making it a pleasant one.

Louise Eriksson – Even though many of our conversations have been about the darkest of thoughts I have appreciated every moment of them.

Alexander Viktorin – My time at MEB has been much more fun since you started working here (again).

Caroline Weibull – I am not sure that I am smart enough to be able to assess your qualities as a biostatistician, but I am certain that you are the best person I know and a wonderful friend. Thank you for your patience and your help.

Thank you **Nancy Pedersen** and **Henrik Grönberg** for creating a nice research environment at MEB.

And thank you to all current and former PhD-students and colleagues, the MEB administrative staff and IT-group for making the department a pleasant place to work at.

Nils – If it wasn't for all our conversations about life and evolution I do not think that any of this would have happened. As you know I believe your place is in academia and if (or when) you decide to give it a shot I would very much like to work with you.

Sebastian – Thank you for making me feel that my work is interesting and important at times when I have not been so sure myself. And thank you for taking and photoshoping my "movie star" press portrait.

Miriam – I believe that this all started during our three-hour lunches at Danderyds gymnasium. Even though we often talked about science we back then both fantasized about artistic professions and did not think that we would follow in our father's footsteps. And look what happened.

Joel – One informal observation I have made during the last couple of years is that friendship is undeniably influenced by the relationship status of friends. Apparently, in this case birds of feather flock together. You and I tend to view life as a one-person show. Nonetheless, I really appreciate having a friend that I can rely on because you (at least for now) share my view on family planning.

Brother – Thank you for always being on my side and for teaching me the valuable lesson that if you want something done right you had better do it yourself.

Sister – My entire life you have been my backup parent at times when I have needed it the most. Thank you for taking care of me.

Mother – You are the botanist of the family and although I might not as a child have been so enthusiastic about learning plant names I do like my memories of our walks in the forests of Åkersberga. Undoubtedly you are also the reason why I like writing so much.

Father – These are the last words I write before sending this off to the printer and I am thinking about when we took detours in the corridors of Stockholms universitet to look at golden frogs and stuffed falcons, all the research equipment you brought home so that I could perform my own experiments in the bathtub, when you let me use your lab to investigate strange stones I had found in our backyard, my first copy of *On the origin of species* that you gave me and all the countless times during my childhood you have explained difficult things to me with simple words. I know that you have never wanted to influence my choice of profession, but of course you did, and I am grateful for that.

8 REFERENCES

1. Crews, D., *Species diversity and the evolution of behavioral controlling mechanisms.* Integrative Neurobiology of Affiliation, 1997. **807**: p. 1-21.
2. Wiederman, M.W., *Extramarital sex: Prevalence and correlates in a national survey.* Journal of Sex Research, 1997. **34**(2): p. 167-174.
3. Lovejoy, C.O., *The Origin of Man.* Science, 1981. **211**(4480): p. 341-350.
4. Marlowe, F.W., *A critical period for provisioning by Hadza men - Implications for pair bonding.* Evolution and Human Behavior, 2003. **24**(3): p. 217-229.
5. Fuentes, A., *Patterns and trends in primate pair bonds.* International Journal of Primatology, 2002. **23**(5): p. 953-978.
6. Hawkes, K., *Mating, parenting and the evolution of human pair bonds*, in *Kinship and Behavior in Primates*, B. Chapais and C. Berman, Editors. 2004, Oxford University Press: Oxford. p. 443-473.
7. Bailey, J.M., et al., *Do individual differences in sociosexuality represent genetic or environmentally contingent strategies? Evidence from the Australian twin registry.* J Pers Soc Psychol, 2000. **78**(3): p. 537-45.
8. Bricker, J.B., et al., *Genetic and environmental influences on age at sexual initiation in the Colorado Adoption Project.* Behav Genet, 2006. **36**(6): p. 820-32.
9. Mustanski, B., et al., *Sexual behavior in young adulthood: a population-based twin study.* Health Psychol, 2007. **26**(5): p. 610-7.
10. Cherkas, L.F., et al., *Genetic influences on female infidelity and number of sexual partners in humans: a linkage and association study of the role of the vasopressin receptor gene (AVPR1A).* Twin Res, 2004. **7**(6): p. 649-58.
11. Trumbetta, S.L. and I.I. Gottesman, *Endophenotypes for marital status in the NAS-NRC twin registry.* Genetic Influences on Human Fertility and Sexuality, 2000: p. 253-269.
12. Spotts, E.L., et al., *Genetic and environmental influences on marital relationships.* J Fam Psychol, 2004. **18**(1): p. 107-19.
13. Mcgue, M. and D.T. Lykken, *Genetic Influence on Risk of Divorce.* Psychological Science, 1992. **3**(6): p. 368-373.
14. Jockin, V., M. McGue, and D.T. Lykken, *Personality and divorce: a genetic analysis.* J Pers Soc Psychol, 1996. **71**(2): p. 288-99.
15. Acher, R., *Chemistry of the neurohypophysial hormones: an example of molecular evolution*, in *Handbook of Physiology*, E. Knobil and W. Sawyer, Editors. 1974, American Physiological Society: Washington, DC. p. 119-130.
16. Burbach, J.P., L.J. Young, and J. Russell, *Oxytocin: synthesis, secretion, and reproductive functions*, in *Knobil and Neill's Physiology of Reproduction*, J.D. Neill, Editor 2006, Elsevier. p. 3055-3128.
17. Boyd, S.K., C.J. Tyler, and G.J. De Vries, *Sexual dimorphism in the vasotocin system of the bullfrog (Rana catesbeiana).* J Comp Neurol, 1992. **325**(2): p. 313-25.
18. Goodson, J.L. and A.H. Bass, *Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates.* Brain Res Brain Res Rev, 2001. **35**(3): p. 246-65.
19. Wang, Z., et al., *Voles and vasopressin: a review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors.* Prog Brain Res, 1998. **119**: p. 483-99.
20. Bielsky, I.F. and L.J. Young, *Oxytocin, vasopressin, and social recognition in mammals.* Peptides, 2004. **25**(9): p. 1565-74.
21. Young, L.J. and Z. Wang, *The neurobiology of pair bonding.* Nat Neurosci, 2004. **7**(10): p. 1048-54.
22. Cho, M.M., et al., *The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster).* Behav Neurosci, 1999. **113**(5): p. 1071-9.

23. Insel, T.R., Z.X. Wang, and C.F. Ferris, *Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents.* J Neurosci, 1994. **14**(9): p. 5381-92.
24. Schorscher-Petcu, A., A. Dupre, and E. Tribollet, *Distribution of vasopressin and oxytocin binding sites in the brain and upper spinal cord of the common marmoset.* Neuroscience Letters, 2009. **461**(3): p. 217-222.
25. Young, L.J., *Oxcytocin and vasopressin receptors and species-typical social behaviors.* Hormones and Behavior, 1999. **36**(3): p. 212-221.
26. Lim, M.M., et al., *Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene.* Nature, 2004. **429**(6993): p. 754-7.
27. Young, L.J., et al., *Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole.* Nature, 1999. **400**(6746): p. 766-8.
28. Hammock, E.A. and L.J. Young, *Variation in the vasopressin V1a receptor promoter and expression: implications for inter- and intraspecific variation in social behaviour.* Eur J Neurosci, 2002. **16**(3): p. 399-402.
29. Williams, J.R., et al., *Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster).* J Neuroendocrinol, 1994. **6**(3): p. 247-50.
30. Insel, T.R. and T.J. Hulihan, *A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles.* Behav Neurosci, 1995. **109**(4): p. 782-9.
31. Insel, T.R. and L.E. Shapiro, *Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles.* Proc Natl Acad Sci U S A, 1992. **89**(13): p. 5981-5.
32. Young, L.J., et al., *Cellular mechanisms of social attachment.* Horm Behav, 2001. **40**(2): p. 133-8.
33. Ross, H.E., et al., *Variation in Oxytocin Receptor Density in the Nucleus Accumbens Has Differential Effects on Affiliative Behaviors in Monogamous and Polygamous Voles.* Journal of Neuroscience, 2009. **29**(5): p. 1312-1318.
34. Young, L.J., et al., *Species differences in central oxytocin receptor gene expression: comparative analysis of promoter sequences.* J Neuroendocrinol, 1996. **8**(10): p. 777-83.
35. Smith, A.S., et al., *Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, Callithrix penicillata.* Horm Behav, 2009. **57**(2): p. 255-62.
36. Born, J., et al., *Sniffing neuropeptides: a transnasal approach to the human brain.* Nat Neurosci, 2002. **5**(6): p. 514-6.
37. Thibonnier, M., et al., *Structure, sequence, expression, and chromosomal localization of the human V1a vasopressin receptor gene.* Genomics, 1996. **31**(3): p. 327-34.
38. Thibonnier, M., et al., *Study of V(1)-vascular vasopressin receptor gene microsatellite polymorphisms in human essential hypertension.* J Mol Cell Cardiol, 2000. **32**(4): p. 557-64.
39. Kim, S.J., et al., *Transmission disequilibrium testing of arginine vasopressin receptor 1A (AVPR1A) polymorphisms in autism.* Mol Psychiatry, 2002. **7**(5): p. 503-7.
40. Wassink, T.H., et al., *Examination of AVPR1a as an autism susceptibility gene.* Molecular Psychiatry, 2004. **9**(10): p. 968-972.
41. Yirmiya, N., et al., *Association between the arginine vasopressin 1a receptor (AVPR1a) gene and autism in a family-based study: mediation by socialization skills.* Molecular Psychiatry, 2006. **11**(5): p. 488-494.
42. Yang, S.Y., et al., *Family-based association study of microsatellites in the 5 ' flanking region of AVPR1A with autism spectrum disorder in the Korean population.* Psychiatry Research, 2010. **178**(1): p. 199-201.
43. Tansey, K.E., et al., *Functionality of promoter microsatellites of arginine vasopressin receptor 1A (AVPR1A): implications for autism.* Molecular autism, 2011. **2**(1): p. 3.

44. Prichard, Z.M., et al., *AVPR1A and OXTR polymorphisms are associated with sexual and reproductive behavioral phenotypes in humans. Mutation in brief no. 981. Online.* Human mutation, 2007. **28**(11): p. 1150.
45. Meyer-Lindenberg, A., et al., *Genetic variants in AVPR1A linked to autism predict amygdala activation and personality traits in healthy humans.* Molecular Psychiatry, 2009. **14**(10): p. 968-975.
46. Knafo, A., et al., *Individual differences in allocation of funds in the dictator game associated with length of the arginine vasopressin 1a receptor RS3 promoter region and correlation between RS3 length and hippocampal mRNA.* Genes Brain and Behavior, 2008. **7**(3): p. 266-275.
47. Avinun, R., et al., *AVPR1A variant associated with preschoolers' lower altruistic behavior.* PLoS One, 2011. **6**(9): p. e25274.
48. Levin, R., et al., *Association between arginine vasopressin 1a receptor (AVPR1a) promoter region polymorphisms and prepulse inhibition.* Psychoneuroendocrinology, 2009. **34**(6): p. 901-8.
49. Vogel, F., et al., *Variable number of tandem repeat polymorphisms of the arginine vasopressin receptor 1A gene and impulsive aggression in patients with borderline personality disorder.* Psychiatric genetics, 2012. **22**(2): p. 105-6.
50. Wu, S., et al., *Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population.* Biol Psychiatry, 2005. **58**(1): p. 74-7.
51. Jacob, S., et al., *Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism.* Neurosci Lett, 2007. **417**(1): p. 6-9.
52. Lerer, E., et al., *Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition.* Mol Psychiatry, 2008. **13**(10): p. 980-8.
53. Yrigollen, C.M., et al., *Genes controlling affiliative behavior as candidate genes for autism.* Biol Psychiatry, 2008. **63**(10): p. 911-6.
54. Tansey, K.E., et al., *Oxytocin receptor (OXTR) does not play a major role in the aetiology of autism: genetic and molecular studies.* Neurosci Lett, 2010. **474**(3): p. 163-7.
55. Wang, K., et al., *Common genetic variants on 5p14.1 associate with autism spectrum disorders.* Nature, 2009. **459**(7246): p. 528-33.
56. Campbell, D.B., et al., *Association of oxytocin receptor (OXTR) gene variants with multiple phenotype domains of autism spectrum disorder.* J Neurodev Disord, 2011. **3**(2): p. 101-12.
57. Liu, X., et al., *Association of the oxytocin receptor (OXTR) gene polymorphisms with autism spectrum disorder (ASD) in the Japanese population.* J Hum Genet, 2010. **55**(3): p. 137-41.
58. Wermter, A.K., et al., *Evidence for the involvement of genetic variation in the oxytocin receptor gene (OXTR) in the etiology of autistic disorders on high-functioning level.* Am J Med Genet B Neuropsychiatr Genet, 2010. **153B**(2): p. 629-39.
59. Chen, F.S. and S.C. Johnson, *An Oxytocin Receptor Gene Variant Predicts Attachment Anxiety in Females and Autism-Spectrum Traits in Males.* Social Psychological and Personality Science, 2011.
60. Rodrigues, S.M., et al., *Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans.* Proc Natl Acad Sci U S A, 2009. **106**(50): p. 21437-41.
61. Costa, B., et al., *Oxytocin receptor polymorphisms and adult attachment style in patients with depression.* Psychoneuroendocrinology, 2009. **34**(10): p. 1506-14.
62. Park, J., et al., *Evidence that genetic variation in the oxytocin receptor (OXTR) gene influences social cognition in ADHD.* Prog Neuropsychopharmacol Biol Psychiatry, 2010. **34**(4): p. 697-702.
63. Kim, H.S., et al., *Culture, distress, and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking.* Proc Natl Acad Sci U S A, 2010. **107**(36): p. 15717-21.
64. Tost, H., et al., *A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function.* Proc Natl Acad Sci U S A, 2010. **107**(31): p. 13936-41.

65. Bakermans-Kranenburg, M.J. and M.H. van Ijzendoorn, *Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting.* Soc Cogn Affect Neurosci, 2008. **3**(2): p. 128-34.
66. Israel, S., et al., *The oxytocin receptor (OXTR) contributes to prosocial fund allocations in the dictator game and the social value orientations task.* PLoS One, 2009. **4**(5): p. e5535.
67. Inoue, H., et al., *Association Between the Oxytocin Receptor Gene and Amygdala Volume in Healthy Adults.* Biol Psychiatry, 2010.
68. Furman, D.J., M.C. Chen, and I.H. Gotlib, *Variant in oxytocin receptor gene is associated with amygdala volume.* Psychoneuroendocrinology, 2011. **36**(6): p. 891-7.
69. Thompson, R.R., et al., *Sex-specific influences of vasopressin on human social communication.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(20): p. 7889-7894.
70. Guastella, A.J., et al., *Arginine Vasopressin Influences on the Recognition of Social Words.* Biological Psychiatry, 2010. **67**(9): p. 174s-174s.
71. Guastella, A.J., et al., *Intranasal Arginine Vasopressin Enhances the Encoding of Happy and Angry Faces in Humans.* Biological Psychiatry, 2010. **67**(12): p. 1220-1222.
72. Guastella, A.J., et al., *Arginine Vasopressin selectively enhances recognition of sexual cues in male humans.* Psychoneuroendocrinology, 2011. **36**(2): p. 294-297.
73. Jarcho, M.R., et al., *Intranasal Vasopressin Affects Pair-Bonding And Peripheral Gene Expression In Male Callicebus Cupreus.* Genes, brain, and behavior, 2011.
74. Kosfeld, M., et al., *Oxytocin increases trust in humans.* Nature, 2005. **435**(7042): p. 673-6.
75. Zak, P.J., A.A. Stanton, and S. Ahmadi, *Oxytocin increases generosity in humans.* PLoS One, 2007. **2**(11): p. e1128.
76. Theodoridou, A., et al., *Oxytocin and social perception: oxytocin increases perceived facial trustworthiness and attractiveness.* Horm Behav, 2009. **56**(1): p. 128-32.
77. Rimmele, U., et al., *Oxytocin makes a face in memory familiar.* J Neurosci, 2009. **29**(1): p. 38-42.
78. De Dreu, C.K., et al., *The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans.* Science, 2010. **328**(5984): p. 1408-11.
79. Domes, G., et al., *Oxytocin improves "mind-reading" in humans.* Biol Psychiatry, 2007. **61**(6): p. 731-3.
80. Andari, E., et al., *Promoting social behavior with oxytocin in high-functioning autism spectrum disorders.* Proc Natl Acad Sci U S A, 2010. **107**(9): p. 4389-94.
81. Guastella, A.J., et al., *Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders.* Biol Psychiatry, 2010. **67**(7): p. 692-4.
82. Bartz, J.A., et al., *Social effects of oxytocin in humans: context and person matter.* Trends in cognitive sciences, 2011. **15**(7): p. 301-9.
83. Unkelbach, C., A.J. Guastella, and J.P. Forgas, *Oxytocin selectively facilitates recognition of positive sex and relationship words.* Psychological Science, 2008. **19**(11): p. 1092-4.
84. Ditzen, B., et al., *Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict.* Biol Psychiatry, 2009. **65**(9): p. 728-31.
85. Carmichael, M.S., et al., *Plasma oxytocin increases in the human sexual response.* J Clin Endocrinol Metab, 1987. **64**(1): p. 27-31.
86. Murphy, M.R., et al., *Changes in Oxytocin and Vasopressin Secretion during Sexual-Activity in Men.* Journal of Clinical Endocrinology & Metabolism, 1987. **65**(4): p. 738-741.
87. Schneiderman, I., et al., *Oxytocin during the initial stages of romantic attachment: Relations to couples' interactive reciprocity.* Psychoneuroendocrinology, 2012.
88. Snowdon, C.T., et al., *Variation in oxytocin is related to variation in affiliative behavior in monogamous, pairbonded tamarins.* Hormones and Behavior, 2010. **58**(4): p. 614-618.

89. Kleiman, D.G., *Monogamy in Mammals.* Quarterly Review of Biology, 1977. **52**(1): p. 39-69.
90. Wittenberger, J.F. and R.L. Tilson, *The Evolution of Monogamy - Hypotheses and Evidence.* Annual Review of Ecology and Systematics, 1980. **11**: p. 197-232.
91. Komers, P.E. and P.N.M. Brotherton, *Female space use is the best predictor of monogamy in mammals.* Proceedings of the Royal Society of London Series B-Biological Sciences, 1997. **264**(1386): p. 1261-1270.
92. van Schaik, C.P. and P.M. Kappeler, *The evolution of social monogamy in primates*, in *Monogamy – mating strategies and partnerships in birds, humans and other mammals*, U.H. Reichard and C. Boesch, Editors. 2003, Cambridge University Press.: Cambridge.
93. Brotherton, P.N.M. and P.E. Komers, *Mate guarding and the evolution of monogamy in mammals*, in *Monogamy – mating strategies and partnerships in birds, humans and other mammals*, U.H. Reichard and C. Boesch, Editors. 2003, Cambridge University Press.: Cambridge.
94. Sillen-Tullberg, B. and A.P. Moller, *The relationship between concealed ovulation and mating systems in anthropoid primates: a phylogenetic analysis.* The American naturalist, 1993. **141**(1): p. 1-25.
95. Hrdy, S.B., *Infanticide among Animals - Review, Classification, and Examination of the Implications for the Reproductive Strategies of Females.* Ethology and Sociobiology, 1979. **1**(1): p. 13-40.
96. Kempenaers, B., G.R. Verheyen, and A.A. Dhondt, *Mate Guarding and Copulation Behavior in Monogamous and Polygynous Blue Tits - Do Males Follow a Best-of-a-Bad-Job Strategy.* Behavioral Ecology and Sociobiology, 1995. **36**(1): p. 33-42.
97. Buss, D.M., et al., *Sex-Differences in Jealousy - Evolution, Physiology, and Psychology.* Psychological Science, 1992. **3**(4): p. 251-255.
98. Winslow, J.T., et al., *A Role for Central Vasopressin in Pair Bonding in Monogamous Prairie Voles.* Nature, 1993. **365**(6446): p. 545-548.
99. Aragona, B.J., et al., *Opposite modulation of social attachment by D1- and D2-type dopamine receptor activation in nucleus accumbens shell.* Horm Behav, 2003(44): p. 37.
100. Aragona, B.J., et al., *Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds.* Nature Neuroscience, 2006. **9**(1): p. 133-139.
101. Darwin, C., *The Descent of Man and Selection in Relation to Sex*1871, London: John Murray.
102. Hedrick, A.V. and E.J. Temeles, *The Evolution of Sexual Dimorphism in Animals - Hypotheses and Tests.* Trends in Ecology & Evolution, 1989. **4**(5): p. 136-138.
103. Shine, R., *Ecological Causes for the Evolution of Sexual Dimorphism - a Review of the Evidence.* Quarterly Review of Biology, 1989. **64**(4): p. 419-461.
104. Andersson, M., *Sexual Selection* 1994, Princeton: Princeton Univ Press.
105. Lande, R., *Sexual Dimorphism, Sexual Selection, and Adaptation in Polygenic Characters.* Evolution, 1980. **34**(2): p. 292-305.
106. Slatkin, M., *Ecological Causes of Sexual Dimorphism.* Evolution, 1984. **38**(3): p. 622-630.
107. Neiderhiser, J.M., et al., *Father-adolescent relationships and the role of genotype-environment correlation.* J Fam Psychol, 2007. **21**(4): p. 560-71.
108. Ganiban, J.M., et al., *Can parent personality explain links between marital satisfaction and parenting?* Journal of Family Psychology, 2009. **23**(5): p. 646-660.
109. Walum, H., et al., *Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans.* Proc Natl Acad Sci U S A, 2008. **105**(37): p. 14153-6.
110. Spanier, G.B., *Measuring Dyadic Adjustment: New scales for Assessing the Quality of Marriage and Similar Dyads.* Journal of Marriage and the Family, 1976. **38**(1): p. 15-28.
111. Simpson, J.A., *Influence of attachment styles on romantic relationships.* Journal of Personality and Social Psychology, 1990. **59**(5): p. 971-980.

112. Booth, A., D. Johnson, and J.N. Edwards, *Measuring Marital Instability.* Journal of Marriage and the Family, 1983. **45**(2): p. 387-394.
113. Lichtenstein, P., et al., *The Swedish Twin Study of CHild and Adolescent Development: The TCHAD-Study.* Twin Research and Human Genetics, 2007. **10**(1): p. 67-73.
114. Plomin, R., et al., *Nature and Nurture - Genetic Contributions to Measures of the Family Environment.* Developmental Psychology, 1994. **30**(1): p. 32-43.
115. Hetherington, E.M., et al., *Coping with Marital Transitions - a Family Systems Perspective.* Monographs of the Society for Research in Child Development, 1992. **57**(2-3): p. R5-&.
116. Achenbach, T.M., *Manual for the Child Behavior Checklist/ 4-18 and 1991 Profile*1993, University of Vermont: Burlington.
117. Lichtenstein, P., et al., *The Swedish Twin Registry in the third millennium: an update.* Twin Res Hum Genet, 2006. **9**(6): p. 875-82.
118. Hansson, S.L., et al., *Psychiatric telephone interview with parents for screening of childhood autism - tics, attention-deficit hyperactivity disorder and other comorbidities (A-TAC): preliminary reliability and validity.* Br J Psychiatry, 2005. **187**: p. 262-7.
119. Larson, T., et al., *The autism--tics, AD/HD and other comorbidities inventory (A-TAC): further validation of a telephone interview for epidemiological research.* BMC Psychiatry, 2010. **10**: p. 1.
120. Harris, C.R., *Factors Associated with Jealousy Over Real and Imagined Infidelity: an Examination of the Social-Cognitive and Evolutionary Psychology Perspectives.* Psychology of Women Quarterly, 2003. **27**(4): p. 319-329.
121. Imhoff, R., et al., *Viewing Time Effects Revisited: Prolonged Response Latencies for Sexually Attractive Targets Under Restricted Task Conditions.* Archives of Sexual Behavior, 2010. **39**(6): p. 1275-1288.
122. Eckel, C.C. and P.J. Grossman, *Altruism in anonymous dictator games.* Games and Economic Behavior, 1996. **16**(2): p. 181-191.
123. Williams, K.D. and B. Jarvis, *Cyberball: A program for use in research on interpersonal ostracism and acceptance.* Behavior Research Methods, 2006. **38**(1): p. 174-180.
124. Zadro, L., K.D. Williams, and R. Richardson, *How low can you go? Ostracism by a computer is sufficient to lower self-reported levels of belonging, control, self-esteem, and meaningful existence.* Journal of Experimental Social Psychology, 2004. **40**(4): p. 560-567.
125. Wassink, T.H., et al., *Examination of AVPR1a as an autism susceptibility gene.* Mol Psychiatry, 2004. **9**(10): p. 968-72.
126. Meyer-Lindenberg, A., et al., *Genetic variants in AVPR1A linked to autism predict amygdala activation and personality traits in healthy humans.* Mol Psychiatry, 2008.
127. Plomin, R., et al., *Behavioral genetics*2008, New York: Worth publishers.
128. Neale, M.C., *Mx: Statistical Modeling: User's guide*. 5th ed1999, Richmond, VA: Department of Human Genetics, Medical College of Virginia.
129. Hallerod, S.L., et al., *The Autism--Tics, AD/HD and other Comorbidities (A-TAC) telephone interview: convergence with the Child Behavior Checklist (CBCL).* Nord J Psychiatry, 2010. **64**(3): p. 218-24.
130. Edlund, J.E. and B.J. Sagarin, *Sex differences in jealousy: Misinterpretation of nonsignificant results as refuting the theory.* Personal Relationships, 2009. **16**(1): p. 67-78.
131. Sagarin, B.J., *Reconsidering evolved sex differences in jealousy: Comment on Harris (2003).* Personality and Social Psychology Review, 2005. **9**(1): p. 62-75.
132. Insel, T.R., *The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior.* Neuron, 2010. **65**(6): p. 768-79.
133. Devries, G.J., et al., *Effects of Androgens and Estrogens on the Vasopressin and Oxytocin Innervation of the Adult-Rat Brain.* Brain Research, 1986. **399**(2): p. 296-302.
134. Ukkola, L.T., et al., *Musical Aptitude Is Associated with AVPR1A-Haplotypes.* PLoS One, 2009. **4**(5).

135. Walum, H., et al., *Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior.* Biological psychiatry, 2012. **71**(5): p. 419-26.
136. Venkatesh, B., et al., *Transgenic rats reveal functional conservation of regulatory controls between the Fugu isotocin and rat oxytocin genes.* Proceedings of the National Academy of Sciences of the United States of America, 1997. **94**(23): p. 12462-12466.
137. Gilligan, P., S. Brenner, and B. Venkatesh, *Neurone-specific expression and regulation of the pufferfish isotocin and vasotocin genes in transgenic mice.* Journal of Neuroendocrinology, 2003. **15**(11): p. 1027-1036.
138. Donaldson, Z.R. and L.J. Young, *Oxytocin, vasopressin, and the neurogenetics of sociality.* Science, 2008. **322**(5903): p. 900-4.
139. Mizuno, J. and N. Takeda, *Phylogenetic Detection of Oxytocin-Immunoreactive and Vasopressin-Immunoreactive Cells in Invertebrates.* Zoological Science, 1986. **3**(6): p. 1080-1080.
140. Takuwa-Kuroda, K., et al., *Octopus, which owns the most advanced brain in invertebrates, has two members of vasopressin/oxytocin superfamily as in vertebrates.* Regulatory Peptides, 2003. **115**(2): p. 139-49.
141. Grimmelikhuijzen, C.J.P., K. Dierickx, and G.J. Boer, *Oxytocin Vasopressin-Like Immunoreactivity Is Present in the Nervous-System of Hydra.* Neuroscience, 1982. **7**(12): p. 3191-3199.
142. De Vries, G.J. and G.C. Panzica, *Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: Different mechanisms, similar endpoints.* Neuroscience, 2006. **138**(3): p. 947-955.
143. Fink, S., L. Excoffier, and G. Heckel, *Mammalian monogamy is not controlled by a single gene.* Proc Natl Acad Sci U S A, 2006. **103**(29): p. 10956-60.
144. Young, L.J. and E.A.D. Hammock, *On switches and knobs, microsatellites and monogamy.* Trends in Genetics, 2007. **23**(5): p. 209-212.
145. Insel, T.R., Z.X. Wang, and C.F. Ferris, *Patterns of Brain Vasopressin Receptor Distribution Associated with Social-Organization in Microtine Rodents.* Journal of Neuroscience, 1994. **14**(9): p. 5381-5392.
146. Ophir, A.G., J.O. Wolff, and S.M. Phelps, *Variation in neural V1aR predicts sexual fidelity and space use among male prairie voles in semi-natural settings.* Proc Natl Acad Sci U S A, 2008. **105**(4): p. 1249-54.
147. Phelps, S.M., *From endophenotypes to evolution: social attachment, sexual fidelity and the avpr1a locus.* Current Opinion in Neurobiology, 2010. **20**(6): p. 795-802.
148. Hammock, E.A. and L.J. Young, *Microsatellite instability generates diversity in brain and sociobehavioral traits.* Science, 2005. **308**(5728): p. 1630-4.
149. Hammond, R.L., et al., *Mating system and avpr1a promoter variation in primates.* Biology Letters, 2008. **4**(4): p. 375-378.
150. Hopkins, W.D., Z.R. Donaldson, and L.J. Young, *A Polymorphic Indel Containing the RS3 Microsatellite in the 5' Flanking Region of the Vasopressin V1a Receptor Gene is Associated with Chimpanzee (Pan troglodytes) Personality.* Genes, brain, and behavior, 2012.
151. Lim, M.M. and L.J. Young, *Neuropeptidergic regulation of affiliative behavior and social bonding in animals.* Hormones and behavior, 2006. **50**(4): p. 506-17.
152. Pedersen, C.A., et al., *Oxytocin Induces Maternal-Behavior in Virgin Female Rats.* Science, 1982. **216**(4546): p. 648-650.
153. Mccarthy, M.M., *Oxytocin Inhibits Infanticide in Female House Mice (Mus-Domesticus).* Hormones and Behavior, 1990. **24**(3): p. 365-375.
154. Kendrick, K.M., E.B. Keverne, and B.A. Baldwin, *Intracerebroventricular Oxytocin Stimulates Maternal-Behavior in the Sheep.* Neuroendocrinology, 1987. **46**(1): p. 56-61.
155. Olazabal, D.E. and L.J. Young, *Oxytocin receptors in the nucleus accumbens facilitate "spontaneous" maternal behavior in adult female prairie voles.* Neuroscience, 2006. **141**(2): p. 559-568.
156. Gordon, I., et al., *Oxytocin and the Development of Parenting in Humans.* Biological Psychiatry, 2010. **68**(4): p. 377-382.
157. Fisher, R.A., *The Genetical Theory of Natural Selection*1930, Oxford: Oxford University Press.

158. Edwards, A.W.F., *The Fundamental Theorem of Natural-Selection.* Biological Reviews of the Cambridge Philosophical Society, 1994. **69**(4): p. 443-474.
159. Kimura, M., *The Neutral Theory of Molecular Evolution*1983, Cambridge: Cambridge University Press.
160. Haldane, J.B., *A Mathematical Theory of Natural and Artificial Selection Part V. Selection and Mutation.* Proc. Cambridge Philos. Soc., 1927. **23**: p. 838–844.
161. Kalmus, H., *Adaptative and selective responses of a population of Drosophila melanogaster containing e and e plus to differences in temperature, humidity and to selection for developmental speed.* Journal of Genetics, 1945. **47**(1): p. 58-63.
162. Williams, G.C., *Pleiotropy, Natural-Selection, and the Evolution of Senescence.* Evolution, 1957. **11**(4): p. 398-411.
163. Charlesworth, D. and B. Charlesworth, *Sex-Differences in Fitness and Selection for Centric Fusions between Sex-Chromosomes and Autosomes.* Genetical Research, 1980. **35**(2): p. 205-214.
164. Levins, R., *Evolution in Changing Environments*1968, New Jersey: Princeton University Press.
165. Allen, J.A. and B.C. Clarke, *Frequency-Dependent Selection - Homage to Poulton,E.B.* Biological Journal of the Linnean Society, 1984. **23**(1): p. 15-18.
166. Roberts, R.L., et al., *Cooperative breeding and monogamy in prairie voles: Influence of the sire and geographical variation.* Animal Behaviour, 1998. **55**: p. 1131-1140.
167. Cushing, B.S. and K.M. Kramer, *Microtines: a model system for studying the evolution and regulation of social monogamy.* Acta Theriologica Sinica, 2005. **25**: p. 182-199.
168. Mcguire, B., T. Pizzuto, and L.L. Getz, *Potential for Social-Interaction in a Natural-Population of Prairie Voles (Microtus-Ochrogaster).* Canadian Journal of Zoology-Revue Canadienne De Zoologie, 1990. **68**(2): p. 391-398.
169. Gangestad, S.W. and J.A. Simpson, *Toward an Evolutionary History of Female Sociosexual Variation.* Journal of Personality, 1990. **58**(1): p. 69-96.
170. Gangestad, S.W. and J.A. Simpson, *The evolution of human mating: Trade-offs and strategic pluralism.* Behavioral and Brain Sciences, 2000. **23**(4): p. 573-+.
171. Maner, J.K., D.A. Rouby, and G.C. Gonzaga, *Automatic inattention to attractive alternatives: the evolved psychology of relationship maintenance.* Evolution and Human Behavior, 2008. **29**(5): p. 343-349.
172. Harmon-Jones, E., C.K. Peterson, and C.R. Harris, *Jealousy: novel methods and neural correlates.* Emotion, 2009. **9**(1): p. 113-7.
173. Harris, C.R., *Sexual and romantic jealousy in heterosexual and homosexual adults.* Psychological Science, 2002. **13**(1): p. 7-12.
174. DeSteno, D.A. and P. Salovey, *Evolutionary origins of sex differences in jealousy? Questioning the ''fitness'' of the model.* Psychological Science, 1996. **7**(6): p. 367-372.