Statistics and Studies of Transsexuals

Collected and Organized by a Transsexual Man

Index

Mental Health

What We Know | What does the scholarly research say about the effect of gender transition on transgender well-being? | What We Know

- Systematic Literature Review, compiled by researchers at Cornell University, 72 studies linked and cited within this PDF.

Mental Health in Transitioning Adults

- Quality of life of individuals with and without facial feminization surgery or gender reassignment surgery
    - "Mental health-related quality of life was statistically diminished ($P < 0.05$) in transgendered women without surgical intervention compared to the general female population and transwomen who had gender reassignment surgery (GRS), facial feminization surgery (FFS), or both. There was no statistically significant difference in the mental health-related quality of life among transgendered women who had GRS, FFS, or both. **Participants who had FFS scored statistically higher ($P < 0.01$) than those who did not in the FFS outcomes evaluation.**"
- Male-to-female transitions: Implications for occupational performance, health, and life satisfaction: Les transitions homme-femme : Conséquences en matière de rendement occupationnel, de santé et de satisfaction face à la vie - Michal Avrech Bar, Tal Jarus, Mineko Wada, Leora Rechtman, Einav Noy, 2016
    - "The results indicate lower performance scores for the transgender women. In addition, occupational settings and group membership (transgender and cisgender groups) were found to be predictors of life satisfaction."
- Socio-demographic variables, clinical features and the role of pre-assessment cross-sex hormones in older trans people
    - "The sex ratio of trans females aged 50 years and older compared to trans males was 23.7:1. Trans males were removed for the analysis due to their small number (n=3). Participants included 71 trans females over the age of 50, of whom the vast majority were white, employed or retired, divorced and had children. **Trans females on CHT that came out as trans and transitioned at an earlier age, were significantly less anxious, reported higher levels of self-esteem and presented with less socialization problems.** When controlling for socialization problems, differences in levels of anxiety but not self-esteem, remained."
- Gender-Related Victimization, Perceived Social Support, and Predictors of Depression Among Transgender Australians
    - "This study examined mental health outcomes, gender-related victimization, perceived social support, and predictors of depression among 243 transgender Australians (n = 83 assigned female at birth, n = 160 assigned male at birth). Overall, 69% reported at least 1 instance of victimization, 59% endorsed depressive symptoms, and 44% reported a

previous suicide attempt. Social support emerged as the most significant predictor of depressive symptoms (p >.05), whereby persons endorsing higher levels of overall perceived social support tended to endorse lower levels of depressive symptoms. Second to social support, persons who endorsed having had some form of gender affirmative surgery were significantly more likely to present with lower symptoms of depression. Contrary to expectations, victimization did not reach significance as an independent risk factor of depression (p =.053). The pervasiveness of victimization, depression, and attempted suicide represents a major health concern and highlights the need to facilitate culturally sensitive health care provision."

- Transgender Emotional and Coping Processes: Facilitative and Avoidant Coping Throughout Gender Transitioning - Stephanie L. Budge, Sabra L. Katz-Wise, Esther N. Tebbe, Kimberly AS Howard, Carrie L. Schneider, Adriana Rodriguez, 2013
    - "Eighteen transgender-identified individuals participated in semi-structured interviews regarding emotional and coping processes throughout their gender transition. The authors used grounded theory to conceptualize and analyze the data. There were three distinct phases through which the participants described emotional and coping experiences: (a) pretransition, (b) during the transition, and (c) posttransition. Five separate themes emerged, including descriptions of coping mechanisms, emotional hardship, lack of support, positive social support, and affirmative emotional experiences. The authors developed a model to describe the role of coping mechanisms and support experienced throughout the transition process. As participants continued through their transitions, emotional hardships lessened and they used facilitative coping mechanisms that in turn led to affirmative emotional experiences. T**he results of this study are indicative of the importance of guiding transgender individuals through facilitative coping experiences and providing social support throughout the transition process. Implications for counselors and for future research are discussed.**"
- WHOQOL-100 Before and After Sex Reassignment Surgery in Brazilian Male-to-Female Transsexual Individuals
    - "The participants showed significant improvement after SRS in domains II (psychological) and IV (social relationships) of the WHOQOL-100. In contrast, domains I (physical health) and III (level of independence) were significantly worse after SRS. Individuals who underwent additional surgery had a decrease in quality of life reflected in domains II and IV. During statistical analysis, all results were controlled for variations in demographic characteristics, without significant results."
- Quality of life and hormones after sex reassignment surgery
    - "The QoL and the quality of body image scores in transpeople were not statistically different from the matched control groups' ones. In the sexual life subscale, transwomen's scores were similar to biological women's ones, whereas transmen's scores were statistically lower than biological men's ones (P = 0.003). The quality of sexual life scored statistically lower in transmen than in transwomen (P = 0.048). **A significant inverse relationship between LH and body image and between LH and quality of sexual life was found.**"
- Transsexual patients' psychiatric comorbidity and positive effect of cross-sex hormonal treatment on mental health: Results from a longitudinal study
    - "The aim of the present study was to evaluate the presence of psychiatric diseases/symptoms in transsexual patients and to compare psychiatric distress related

to the hormonal intervention in a one year follow-up assessment. We investigated 118 patients before starting the hormonal therapy and after about 12 months. We used the SCID-I to determine major mental disorders and functional impairment. We used the Zung Self-Rating Anxiety Scale (SAS) and the Zung Self-Rating Depression Scale (SDS) for evaluating self-reported anxiety and depression. We used the Symptom Checklist 90-R (SCL-90-R) for assessing self-reported global psychological symptoms. Seventeen patients (14%) had a DSM-IV-TR axis I psychiatric comorbidity. At enrollment the mean SAS score was above the normal range. The mean SDS and SCL-90-R scores were on the normal range except for SCL-90-R anxiety subscale. When treated, patients reported lower SAS, SDS and SCL-90-R scores, with statistically significant differences. **Psychiatric distress and functional impairment were present in a significantly higher percentage of patients before starting the hormonal treatment than after 12 months (50% vs. 17% for anxiety; 42% vs. 23% for depression; 24% vs. 11% for psychological symptoms; 23% vs. 10% for functional impairment).** The results revealed that the majority of transsexual patients have no psychiatric comorbidity, suggesting that transsexualism is not necessarily associated with severe comorbid psychiatric findings. The condition, however, seemed to be associated with subthreshold anxiety/depression, psychological symptoms and functional impairment. Moreover, treated patients reported less psychiatric distress. **Therefore, hormonal treatment seemed to have a positive effect on transsexual patients' mental health.**"

- Hormonal Treatment Reduces Psychobiological Distress in Gender Identity Disorder, Independently of the Attachment Style
    - "At enrollment, transsexuals reported elevated CAR; their values were out of normal. They expressed higher perceived stress and more attachment insecurity, with respect to normative sample data. **When treated with hormone therapy, transsexuals reported significantly lower CAR ($P < 0.001$), falling within the normal range for cortisol levels. Treated transsexuals showed also lower perceived stress ($P < 0.001$), with levels similar to normative samples.** The insecure attachment styles were associated with higher CAR and perceived stress in untreated transsexuals ($P < 0.01$). Treated transsexuals did not express significant differences in CAR and perceived stress by attachment."
- The Effects of Hormonal Gender Affirmation Treatment on Mental Health in Female-to-Male Transsexuals
    - "Hormonal interventions are an often-sought option for transgender individuals seeking to medically transition to an authentic gender. Current literature stresses that the effects and associated risks of hormone regimens should be monitored and well understood by health care providers (Feldman & Bockting, 2003). However, the positive psychological effects following hormone replacement therapy as a gender affirming treatment have not been adequately researched. This study examined the relationship of hormone replacement therapy, specifically testosterone, with various mental health outcomes in an Internet sample of more than 400 self-identified female-to-male transsexuals. **Results of the study indicate that female-to-male transsexuals who receive testosterone have lower levels of depression, anxiety, and stress, and higher levels of social support and health related quality of life.** Testosterone use was not related to problems with drugs, alcohol, or suicidality. **Overall findings provide clear evidence that HRT is associated with improved mental health outcomes in female-to-male transsexuals.**"

- A Prospective Study on Sexual Function and Mood in Female-to-Male Transsexuals During Testosterone Administration and After Sex Reassignment Surgery
    - "Testosterone administration in female-to-male transsexual subjects aims to develop and maintain the characteristics of the desired sex. Very little data exists on its effects on sexuality of female-to-male transsexuals. The aim of this study was to evaluate sexual function and mood of female-to-male transsexuals from their first visit, throughout testosterone administration and after sex reassignment surgery. Participants were 50 female-to-male transsexual subjects who completed questionnaires assessing sexual parameters and mood. The authors measured reproductive hormones and hematological parameters. **The results suggest a positive effect of testosterone treatment on sexual function and mood in female-to-male transsexual subjects.**"
- Effects of Testosterone Treatment and Chest Reconstruction Surgery on Mental Health and Sexuality in Female-To-Male Transgender People
    - "*Results:* Cross-sectional analysis using a between-subjects multivariate analysis of variance showed that participants who were receiving testosterone endorsed fewer symptoms of anxiety and depression as well as less anger than the untreated group. **Participants who had CRS in addition to testosterone reported less body dissatisfaction than both the testosterone-only or the untreated groups.** Furthermore, participants who were injecting testosterone on a weekly basis showed significantly less anger compared with those injecting every other week. In qualitative reports, more than 50% of participants described increased sexual attraction to nontransgender men after taking testosterone."
- The role of gender affirmation in psychological well-being among transgender women
    - "High prevalence of psychological distress, including greater depression, lower self-esteem, and suicidal ideation, has been documented across numerous samples of transgender women and has been attributed to high rates of discrimination and violence. According to the gender affirmation framework (Sevelius, 2013), access to sources of gender-affirmative support can offset such negative psychological effects of social oppression. However, critical questions remain unanswered in regards to how and which aspects of gender affirmation are related to psychological well-being. The aims of this study were to investigate the associations between three discrete areas of gender affirmation (psychological, medical, and social) and participants' reports of psychological well-being. A community sample of 573 transgender women with a history of sex work completed a one-time self-report survey that assessed demographic characteristics, gender affirmation, and mental health outcomes. In multivariate models, we found that social, psychological, and medical gender affirmation were significant predictors of lower depression and higher self-esteem while no domains of affirmation were significantly associated with suicidal ideation. **Findings support the need for accessible and affordable transitioning resources for transgender women in order to promote better quality of life among an already vulnerable population.** As the gender affirmation framework posits, the personal experience of feeling affirmed as a transgender person results from individuals' subjective perceptions of need along multiple dimensions of gender affirmation. Personalized assessment of gender affirmation may thus be a useful component of counseling and service provision for transgender women."
- Hormone-treated transsexuals report less social distress, anxiety and depression

    - "The mean SADS and HADS scores were in the normal range except for the HAD-Anxiety subscale (HAD-A) on the non-treated transsexual group. SADS, HAD-A, and HAD-Depression (HAD-D) mean scores were significantly higher among patients who had not begun cross-sex hormonal treatment compared with patients in hormonal treatment (F = 4.362, p = .038; F = 14.589, p = .001; F = 9.523, p = .002 respectively). **Similarly, current symptoms of anxiety and depression were present in a significantly higher percentage of untreated patients than in treated patients (61% vs. 33% and 31% vs. 8% respectively).**"
- Determinants of quality of life in Spanish transsexuals attending a gender unit before genital sex reassignment surgery
    - "Mean scores of all QoL domains ranged from 55.44 to 63.51. **Linear regression analyses revealed that undergoing cross-sex hormonal treatment, having family support, and having an occupation were associated with a better QoL for all transsexuals**. FM transsexuals have higher social domain QoL scores than MF transsexuals. The model accounts for 20.6 % of the variance in the physical, 32.5 % in the psychological, 21.9 % in the social, and 20.1 % in the environment domains, and 22.9 % in the global QoL factor."
- Is Hormonal Therapy Associated with Better Quality of Life in Transsexuals? A Cross-Sectional Study
    - "The mean age of the total sample was 34.7 years, and the sex ratio was 1:1. Forty-four (72.1%) of the participants received hormonal therapy. **Hormonal therapy and depression were independent predictive factors of the SF-36 mental composite score. Hormonal therapy was significantly associated with a higher QoL, while depression was significantly associated with a lower QoL.** Transsexuals' QoL, independently of hormonal status, did not differ from the French age- and sex-matched controls except for two subscales of the SF-36 questionnaire: role physical (lower scores in transsexuals) and general health (lower scores in controls)."
- Hormonal Therapy Is Associated With Better Self-esteem, Mood, and Quality of Life in Transsexuals
    - "Few studies have assessed the role of cross-sex hormones on psychological outcomes during the period of hormonal therapy preceding sex reassignment surgery in transsexuals. The objective of this study was to assess the relationship between hormonal therapy, self-esteem, depression, quality of life (QoL), and global functioning. This study incorporated a cross-sectional design. The inclusion criteria were diagnosis of gender identity disorder (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision) and inclusion in a standardized sex reassignment procedure. The outcome measures were self-esteem (Social Self-Esteem Inventory), mood (Beck Depression Inventory), QoL (Subjective Quality of Life Analysis), and global functioning (Global Assessment of Functioning). Sixty-seven consecutive individuals agreed to participate. Seventy-three percent received hormonal therapy. **Hormonal therapy was an independent factor in greater self-esteem, less severe depression symptoms, and greater "psychological-like" dimensions of QoL. These findings should provide pertinent information for health care providers who consider this period as a crucial part of the global sex reassignment procedure.**"
- Satisfaction With Male-to-Female Gender Reassignment Surgery (21.11.2014)

    - "119 (46.9%) of the patients filled out and returned the questionnaires, at a mean of 5.05 years after surgery (standard deviation 1.61 years, range 1–7 years). **90.2% said their expectations for life as a woman were fulfilled postoperatively.** 85.4% saw themselves as women. 61.2% were satisfied, and 26.2% very satisfied, with their outward appearance as a woman; 37.6% were satisfied, and 34.4% very satisfied, with the functional outcome. 65.7% said they were satisfied with their life as it is now."
- Effects of Different Steps in Gender Reassignment Therapy on Psychopathology: A Prospective Study of Persons with a Gender Identity Disorder
    - "**A difference in SCL-90 overall psychoneurotic distress was observed at the different points of assessments (*P* = 0.003), with the most prominent decrease occurring after the initiation of hormone therapy (*P* < 0.001). Significant decreases were found in the subscales such as anxiety, depression, interpersonal sensitivity, and hostility.** Furthermore, the SCL-90 scores resembled those of a general population after hormone therapy was initiated. Analysis of the psychosocial variables showed no significant differences between pre- and postoperative assessments."
- ORIGINAL RESEARCH—INTERSEX AND GENDER IDENTITY DISORDERS: A Report from a Single Institute's 14-Year Experience in Treatment of Male-to-Female Transsexuals
    - "Average age was 31 years old. Seventy-two percent had a high educational level, and 63% were steadily employed. Half of the patients had contemplated suicide at some time in their lives before surgery and 4% had actually attempted suicide. Family and colleague emotional support levels were satisfactory. All patients had been adequately informed of surgical procedure beforehand. Eighty-nine percent engaged in postsurgical sexual activities. Seventy-five percent had a more satisfactory sex life after SRS, with main complications being pain during intercourse and lack of lubrication. Seventy-eight percent were satisfied with their neovagina's esthetic appearance, whereas only 56% were satisfied with depth. **Almost all of the patients were satisfied with their new sexual status and expressed no regrets**."
- Testosterone treatment and MMPI-2 improvement in transgender men: a prospective controlled study
    - "**Statistically significant changes in MMPI-2 scale scores were found at 3-month follow-up after initiating testosterone treatment relative to baseline for transgender men compared with female controls** (female template): reductions in Hypochondria ($p < .05$), Depression ($p < .05$), Hysteria ($p < .05$), and Paranoia ($p < .01$); and increases in Masculinity-Femininity scores ($p < .01$). Gender × Time interaction effects were found for Hysteria ($p < .05$) and Paranoia ($p < .01$) relative to female controls (female template) and for Hypochondria ($p < .05$), Depression ($p < .01$), Hysteria ($p < .01$), Psychopathic Deviate ($p < .05$), Paranoia ($p < .01$), Psychasthenia ($p < .01$), and Schizophrenia ($p < .01$) compared with male controls (male template). In addition, the proportion of transgender men presenting with co-occurring psychopathology significantly decreased from baseline compared with 3-month follow-up relative to controls ($p < .05$)."
- Body image and transsexualism
    - "We found that preoperative transsexual patients were insecure and felt unattractive because of concerns about their body image. **However, postoperative transsexual patients scored high on attractiveness and self-confidence. Furthermore, postoperative transsexual patients showed low scores for insecurity and concerns about their body.**"

- Factors predictive of regret in sex reassignment - Landén - 1998 - Acta Psychiatrica Scandinavica
    - "The objective of this study was to evaluate the features and calculate the frequency of sex-reassigned subjects who had applied for reversal to their biological sex, and to compare these with non-regretful subjects. An inception cohort was retrospectively identified consisting of all subjects with gender identity disorder who were approved for sex reassignment in Sweden during the period 1972-1992. The period of time that elapsed between the application and this evaluation ranged from 4 to 24 years. The total cohort consisted of 218 subjects. The results showed that 3.8% of the patients who were sex reassigned during 1972-1992 regretted the measures taken. The cohort was subdivided according to the presence or absence of regret of sex reassignment, and the two groups were compared. The results of logistic regression analysis indicated that two factors predicted regret of sex reassignment, namely lack of support from the patient's family, and the patient belonging to the non-core group of transsexuals. **In conclusion, the results show that the outcome of sex reassignment has improved over the years. However, the identified risk factors indicate the need for substantial efforts to support the families and close friends of candidates for sex reassignment.**"
- Factors Associated with Satisfaction or Regret Following Male-to-Female Sex Reassignment Surgery
    - "This study examined factors associated with satisfaction or regret following sex reassignment surgery (SRS) in 232 male-to-female transsexuals operated on between 1994 and 2000 by one surgeon using a consistent technique. Participants, all of whom were at least 1-year postoperative, completed a written questionnaire concerning their experiences and attitudes. **Participants reported overwhelmingly that they were happy with their SRS results and that SRS had greatly improved the quality of their lives. None reported outright regret and only a few expressed even occasional regret. Dissatisfaction was most strongly associated with unsatisfactory physical and functional results of surgery.** Most indicators of transsexual typology, such as age at surgery, previous marriage or parenthood, and sexual orientation, were not significantly associated with subjective outcomes. Compliance with minimum eligibility requirements for SRS specified by the Harry Benjamin International Gender Dysphoria Association was not associated with more favorable subjective outcomes. The physical results of SRS may be more important than preoperative factors such as transsexual typology or compliance with established treatment regimens in predicting postoperative satisfaction or regret."
- Patient-Reported Complications and Functional Outcomes of Male-to-Female Sex Reassignment Surgery
    - "This study examined preoperative preparations, complications, and physical and functional outcomes of male-to-female sex reassignment surgery (SRS), based on reports by 232 patients, all of whom underwent penile-inversion vaginoplasty and sensate clitoroplasty, performed by one surgeon using a consistent technique. Nearly all patients discontinued hormone therapy before SRS and most reported that doing so created no difficulties. Preoperative electrolysis to remove genital hair, undergone by most patients, was not associated with less serious vaginal hair problems. No patients reported rectal-vaginal fistula or deep-vein thrombosis and reports of other significant surgical complications were uncommon. One third of patients, however, reported urinary stream

problems. N**o single complication was significantly associated with regretting SRS. Satisfaction with most physical and functional outcomes of SRS was high; participants were least satisfied with vaginal lubrication, vaginal touch sensation, and vaginal erotic sensation. Frequency of achieving orgasm after SRS was not significantly associated with most general measures of satisfaction.** Later years of surgery, reflecting greater surgeon experience, were not associated with lower prevalence rates for most complications or with better ratings for most physical and functional outcomes of SRS."

- Medical Treatment of Subjects with Gender Identity Disorder: The Experience in an Italian Public Health Center
    - "Hormonal treatment is the main element during the transition program for transpeople. The aim of this paper is to describe the care and treatment of subjects, highlighting both the endocrine-metabolic effects of the hormonal therapy and the quality of life during the first year of cross-sex therapy in an Italian gender team. We studied 83 subjects (56 male-to-female [MtF], 27 female-to-male [FtM]) with hematological and hormonal evaluations every 3 months during the first year of hormonal therapy. MtF persons were treated with 17βestradiol and antiandrogens (cyproterone acetate, spironolactone, dutasteride); FtM persons were treated with transdermal or intramuscular testosterone. The WHO Quality of Life questionnaire was administered at the beginning and 1 year later. Hormonal changes paralleled phenotype modifications with wide variability. **Most of both MtF and FtM subjects reported a statistically significant improvement in body image ($p < 0.05$). In particular, MtF subjects reported a statistically significant improvement in the quality of their sexual life and in the general quality of life ($p < 0.05$) 1 year after treatment initiation.** Cross-sex therapy seems to be free of major risks in healthy subjects under clinical supervision during the first year. Selected subjects show an optimal adaptation to hormone-induced neuropsychological modifications and satisfaction regarding general and sexual life."
- Anxiety and depression in males experiencing gender dysphoria
    - "There was no significant change in anxiety and depression scores in people with gender dysphoria (male to female) pre- and post-operatively."
- Transgender patient satisfaction following reduction mammaplasty
    - "Seventeen patients were identified. The senior author performed bilateral reduction mammaplasties and free nipple grafts in 16 patients and one patient had a Benelli technique reduction. Complications included two haematomas, one wound infection, one wound dehiscence and three patients had hypertrophic scars. Secondary surgery was performed in seven patients and included scar revision, nipple reduction/realignment, dog-ear correction and nipple tattooing. The mean follow-up period after surgery was 10 months (range 2–23 months). Twelve postal questionnaires were completed (response rate 70%). **All respondents expressed satisfaction with their result and no regret.** Seven patients had nipple sensation and nine patients were satisfied with nipple position. All patients thought their scars were reasonable and felt that surgery had improved their self-confidence and social interactions."
- Female-to-male transgender quality of life
    - "Analysis of quality of life health concepts demonstrated statistically significant ($p<0.01$) diminished quality of life among the FTM transgender participants as compared to the US male and female population, particularly in regard to mental health. **FTM transgender**

participants who received testosterone (67%) reported statistically significant higher quality of life scores (*p*<0.01) than those who had not received hormone therapy."

- Societal Implications of Health Insurance Coverage for Medically Necessary Services in the U.S. Transgender Population: A Cost-Effectiveness Analysis
    - "Compared to no health benefits for transgender patients ($23,619; 6.49 QALYs), insurance coverage for medically necessary services came at a greater cost and effectiveness ($31,816; 7.37 QALYs), with an incremental cost-effectiveness ratio (ICER) of $9314/QALY. The budget impact of this coverage is approximately $0.016 per member per month. Although the cost for transitions is $10,000–22,000 and the cost of provider coverage is $2175/year, these additional expenses hold good value for reducing the risk of negative endpoints —HIV, depression, suicidality, and drug abuse. Results were robust to uncertainty. The probabilistic sensitivity analysis showed that provider coverage was cost-effective in 85 % of simulations."
- Study of quality of life for transsexuals after hormonal and surgical reassignment
    - "**The results show that gender reassignment surgery improves the QoL for transsexuals in several different important areas: most are satisfied of their sexual reassignment (28/30), their social (21/30) and sexual QoL (25/30) are improved.** However, there are differences between male-to-female (MtF) and female-to-male (FtM) transsexuals in terms of QoL: FtM have a better social, professional, friendly lifestyles than MtF. Finally, the results of this study did not evidence any influence by certain aspects of the personality, such as extraversion and neuroticism, on the QoL for reassigned subjects."
- Regrets After Sex Reassignment Surgery: Journal of Psychology & Human Sexuality: Vol 5, No 4
    - "Using data draw from the follow-up literature covering the last 30 years, and the author's clinical data on 295 men and women after SRS, an estimation of the number of patients who regretted the operations is made. **Among female-to-male transsexuals after SRS, i.e., in men, no regrets were reported in the author's sample, and in the literature they amount to less than 1%. Among male-to-female transsexuals after SRS, i.e., in women, regrets are reported in 1-1.5%**. Poor differential diagnosis, failure to carry out the real-life- test, and poor surgical results seem to be the main reasons behind the regrets reported in the literature. According to three cases observed by the author in addition to personality traits the lack of proper care in treating the patients played a major role."
- Transsexualism: Treatment Outcome of Compliant and Noncompliant Patients
    - "The objective of the study was a follow-up of the treatment outcome of Finnish transsexuals who sought sex reassignment during the period 1970–2002 and a comparison of the results and duration of treatment of compliant and noncompliant patients. Fifteen male-to-female transsexuals and 17 female-to-male transsexuals who had undergone hormone and surgical treatment and legal sex reassignment in Finland completed a questionnaire on psychosocial data and on their experience with the different phases of clinical assessment and treatment. The changes in their vocational functioning and social and psychic adjustment were used as outcome indicators. The results and duration of the treatment of compliant and noncompliant patients were compared. **The patients benefited significantly from treatment.** The noncompliant patients achieved equally good results as the compliant ones, and did so in a shorter time. A good treatment outcome could be achieved even when the patient had told the assessing psychiatrist a falsified story of his life and sought hormone therapy, genital

surgery, or legal sex reassignment on his own initiative without a recommendation from the psychiatrist. Based on these findings, it is recommended that the doctor-patient relationship be reconsidered and founded on frank cooperation."

- The outcome of sex reassignment surgery in Belgrade: 32 patients of both sexes
    - "Several aspects of the quality of life after sex reassignment surgery in 32 transsexuals of both sexes (22 men, 10 women) were examined. The Belgrade Team for Gender Identity Disorders designed a standardized questionnaire for this purpose. The follow-up period after operation was from 6 months to 4 years, and four aspects of the quality of life were examined: attitude towards the patients' own body, relationships with other people, sexual activity, and occupational functioning. **In most transsexuals, the quality of life was improved after surgery inasmuch as these four aspects are concerned. Only a few transsexuals were not satisfied with their life after surgery.**"
- Patient Satisfaction with Breasts and Psychosocial, Sexual,... : Plastic and Reconstructive Surgery
    - "Thirty-five male-to-female transsexual patients completed the questionnaires. B**REAST-Q subscale median scores (satisfaction with breasts, +59 points; sexual well-being, +34 points; and psychosocial well-being, +48 points) improved significantly ($p < 0.05$) at 4 months postoperatively and later.** No significant change was observed in physical well-being."
- IJ TRANSGENDER - Psychological and Social Function Before and After Phalloplasty
    - "There were significant differences between the populations. The post operative group showed higher depression ratings on the depression subscale of the GHQ. The masculine pre-operative Bem scores were neutral post-operatively as feminine sub-scores increased. There was improved satisfaction with genital appearance post-operatively, but satisfaction with relationships fell, although to a non-significant extent. Most other changes were in the expected direction but did not achieve significance. Transsexuals accepted for phalloplasty have very good psychological health. **Tendency to further improvement is the case after phalloplasty.** Depression is commoner, however, and quality of relationships declines somewhat, perhaps in consequence. Surgeons might advise partners as well as patients of realistic expectations from such surgery."
- Quality of life improves early after gender reassignment surgery in transgender women
    - "On most dimensions of the SF-36 questionnaire, transgender women reported a lower QoL than the general population. **The scores of SF-36 showed a non-significant trend to be lower 5 years post-GRS compared to pre-operatively, a decline consistent with that of the general population.** Self-perceived health compared to 1 year previously rose in the first post-operative year, after which it declined."
- Psychological functions in male-to-female transsexual people before and after surgery
    - "Patients with gender dysphoria (GD) suffer from a constant feeling of psychological discomfort related to their anatomical sex. Gender reassignment surgery (GRS) attempts to release this discomfort. The aim of this study was to compare the functioning of a cohort or patients with GD before and after GRS. We hypothesised that there would be an improvement in the scores of the self-administered SCL-90R following gender reassignment surgery among male-to-female people with gender dysphoria. We studied 40 patients with a DSM-IV diagnosis of Gender Identity Disorder (GID) who attended Leicester Gender Identity Clinic. We compared their functioning as measured by Symptom Check List-90R (SCL-90R) which was administered to 40 randomly selected

male-to-female patients before and within six months after GRS using the same sample as control pre-and post-surgery. There was no significant change in the different sub-scales of the SCL-90R scores in patients with male-to-female GID pre- and within six months post-surgery. **The results of the study showed that GRS had no significant effect on functioning as measured by SCL-90R within six months of surgery.** Our study has the advantage of reducing inter-subject variability by using the same patients as their own control. This study may be limited by the duration of reassessment post-surgery. Further studies with larger sample size and using other psychosocial scales are needed to elucidate on the effectiveness of surgical intervention on psychosocial parameters in patients with GD."

- Hormonal therapy and sex reassignment: A systematic review and meta-analysis of quality of life and psychosocial outcomes
    - "Pooling across studies shows that after sex reassignment, **80% of individuals with GID reported significant improvement in gender dysphoria (95% CI = 68-89%; 8 studies; I 2 = 82%); 78% reported significant improvement in psychological symptoms (95% CI = 56-94%; 7 studies; I 2 = 86%); 80% reported significant improvement in quality of life (95% CI = 72-88%; 16 studies; I 2 = 78%);** and 72% reported significant improvement in sexual function (95% CI = 60-81%; 15 studies; I 2 = 78%)."
- Psychosocial Adjustment to Sex Reassignment Surgery: A Qualitative Examination and Personal Experiences of Six Transsexual Persons in Croatia
    - "**On the DASS, all participants achieved results that fell within the category of normal mood variations.** This finding is also true for both the subscales and the overall results on the CORE-OM questionnaire, where all the results fall under gender specific cutoff scores."
- Transsexualism. Epidemiology, phenomenology, regret after surgery, aetiology, and public attitudes
    - "Transsexualism denotes a condition in which the gender identity-the personal sense of being a man or a woman-contradicts the bodily sex characteristics. This thesis is based on three independent surveys about transsexualism. FIRST, all 233 subjects applying for sex reassignment in Sweden during 1972-1992 were retrospectively examined through medical records. The incidence of applying for sex reassignment was 0.17/100,000 individuals over 15 years of age and per year. The male-to-female (M-F)/female-to-male (F-M) ratio was 1.4/1. With the exception of an incidence peak related to the legislation regulating sex reassignment in the early 1970s, the incidence has remained fairly stable since the first estimates in Sweden in the late 1960s. The M-F (n=134) and F-M (n=99) groups were phenomenologically compared. M-F transsexuals were older, and more often had a history of marriage and children than their F-M counterparts. M-F transsexuals also had more heterosexual experience. F-M transsexuals, on the other hand, more frequently reported cross-gender behaviour in childhood than did M-F transsexuals. It is concluded that transsexualism is manifested differently in males and females. The regret frequency (defined as applying for reversal to the original sex) was 3.8%. Prognostic factors for regret were, 'a poor support from the family', and 'belonging to the secondary group of transsexuals' (denotes people who develop transsexualism only after a significant period of transvestism or homosexuality). SECOND, 28 M-F transsexuals and 30 male controls were investigated. To test the hypothesis that genes coding for proteins involved in the sexual differentiation of the brain influence the

susceptibility of transsexualism, we analysed (1) a tetra nucleotide polymorphism of the aromatase gene, (2) a CAG repeat sequence in the first exon of the gene coding for the androgen receptor, and (3) a CA repeat polymorphism of the estrogen receptor beta gene. Results support the notion that the gender identity is related to the sex steroid-driven sexual differentiation of the brain, and that certain genetic variants of three of the genes critically involved in this process, may enhance the susceptibility for transsexualism.THIRD, a questionnaire comprising questions about attitudes towards transsexualism and transsexuals was mailed to a random national sample (n=998) of Swedish residents, 18-75 years of age. The response rate was 67%. The results showed that a majority supports the possibility for transsexuals to undergo sex reassignment. However, 63% thought that the individual should bear the expenses for it. In addition, a majority supported the transsexuals' right to get married in their new sex, and their right to work with children. Transsexuals' right to adopt and raise children was supported by 43% whereas 41% opposed this. The results indicated that those who believed that transsexualism is caused by psychological factors had a more restrictive view on transsexualism than people who held a biological view."

- Levels of depression in transgender people and its predictors: Results of a large matched control study with transgender people accessing clinical services.
    - "*Results:* Individuals were categorized as having no, possible or probable depressive disorder. Transgender individuals not on CHT had a nearly four-fold increased risk of probable depressive disorder, compared to controls. Older age, lower self-esteem, poorer interpersonal function and less social support predicted depressive disorder. Use of CHT was associated with less depression.
      Conclusion: This study confirms that non-treated transgender individuals have an increased risk of a depressive disorder. Interventions offered alongside gender affirming treatment to develop interpersonal skills, increase self-esteem and improve social support may reduce depression and prepare individuals for a more successful transition."
- Gender-Affirming Hormone Use in Transgender Individuals: Impact on Behavioral Health and Cognition
    - "Although there are some conflicting data, GAHT overwhelmingly seems to have positive psychological effects in both adolescents and adults. Research tends to support that GAHT reduces symptoms of anxiety and depression, lowers perceived and social distress, and improves quality of life and self-esteem in both male-to-female and female-to-male transgender individuals. Clinically, prescribing GAHT can help with gender dysphoria-related mental distress. Thus, timely hormonal intervention represents a crucial tool for improving behavioral wellness in transgender individuals, though effects on cognitive processes fundamental for daily living are unknown. Future research should prioritize better understanding of how GAHT may affect executive functioning."
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Mental Health in Transitioning Youths

- Mental Health and Self-Worth in Socially Transitioned Transgender Youth
    - "Transgender children reported depression and self-worth that did not differ from their matched-control or sibling peers (p = .311), and they reported marginally higher anxiety (p = .076). Compared with national averages, transgender children showed typical rates of

depression (p = .290) and marginally higher rates of anxiety (p = .096). Parents similarly reported that their transgender children experienced more anxiety than children in the control groups (p = .002) and rated their transgender children as having equivalent levels of depression (p = .728)."

- Young Adult Psychological Outcome After Puberty Suppression and Gender Reassignment
    - "After gender reassignment, in young adulthood, the GD was alleviated and psychological functioning had steadily improved. Well-being was similar to or better than same-age young adults from the general population. Improvements in psychological functioning were positively correlated with postsurgical subjective well-being."
- Sex reassignment: outcomes and predictors of treatment for adolescent and adult transsexuals
    - "**After treatment the group was no longer gender dysphoric. The vast majority functioned quite well psychologically, socially and sexually.** Two non-homosexual male-to-female transsexuals expressed regrets. Post-operatively, female-to-male and homosexual transsexuals functioned better in many respects than male-to-female and non-homosexual transsexuals. Eligibility for treatment was largely based upon gender dysphoria, psychological stability, and physical appearance. Male-to-female transsexuals with more psychopathology and cross-gender symptoms in childhood, yet less gender dysphoria at application, were more likely to drop out prematurely. Non-homosexual applicants with much psychopathology and body dissatisfaction reported the worst post-operative outcomes."
- Allowing for transgender youth to transition improves their mental health, study finds
    - Link now leads to an Error page. Access may still be possible through other means.
- Trans kids who socially transition early and who are not subjected to abuse or discrimination are comparable to cisgender children in measures of mental health
    - Link now leads to an Error page. Access may still be possible through other means.

Suicide Rates, Self-Destructive Behavior, and Prevention

- Suicide risk in the UK trans population and the role of gender transition in decreasing suicidal ideation and suicide attempt
    - "The study revealed high rates of suicidal ideation (84 per cent lifetime prevalence) and attempted suicide (48 per cent lifetime prevalence) within this sample. A supportive environment for social transition and timely access to gender reassignment, for those who required it, emerged as key protective factors. Subsequently, gender dysphoria, confusion/denial about gender, fears around transitioning, gender reassignment treatment delays and refusals, and social stigma increased suicide risk within this sample."
- Science AMA Series: I'm Cecilia Dhejne a fellow of the European Committee of Sexual Medicine, from the Karolinska University Hospital in Sweden. I'm here to talk about transgender health, suicide rates, and my often misinterpreted study. Ask me anything! : science
- Intervenable factors associated with suicide risk in transgender persons: a respondent driven sampling study in Ontario, Canada

    - "Among trans Ontarians, 35.1 % (95 % CI: 27.6, 42.5) seriously considered, and 11.2 % (95 % CI: 6.0, 16.4) attempted, suicide in the past year. Social support, reduced transphobia, and having any personal identification documents changed to an appropriate sex designation were associated with large relative and absolute reductions in suicide risk, as was completing a medical transition through hormones and/or surgeries (when needed). Parental support for gender identity was associated with reduced ideation. Lower self-reported transphobia (10thversus 90th percentile) was associated with a 66 % reduction in ideation (RR = 0.34, 95 % CI: 0.17, 0.67), and an additional 76 % reduction in attempts among those with ideation (RR = 0.24; 95 % CI: 0.07, 0.82). This corresponds to potential prevention of 160 ideations per 1000 trans persons, and 200 attempts per 1,000 with ideation, based on a hypothetical reduction of transphobia from current levels to the 10th percentile."
- Suicide Protective Factors Among Trans Adults
    - "All but one participant (*n* = 132) answered all four questions on the SBQ-R. The mean total score was 9.8 (*SD* = 3.7) out of a possible range of 3–18. Although not used in the context of this research, the SBQ-R has two validated cutoff scores indicating risk for suicidal behavior: scores ≥7, validated with undergraduate students and scores ≥8, validated with adult inpatients. Score distributions of the SBQ-R are shown in Table. Preliminary analyses (i.e., *t*-tests and ANOVA) examined whether participants' suicidal behavior scores differed significantly according to demographic variables. Results indicated that suicidal behavior scores did not differ significantly as a function of demographic variables."
- Trans Mental health Study 2012
    - "Suicidal ideation and actual attempts reduced after transition, with 63% thinking about or attempting suicide more before they transitioned and only 3% thinking about or attempting suicide more post-transition."
- Risk Factors for Non-Suicidal Self-Injury Among Trans Youth
    - "A lifetime presence of NSSI was identified in 46.3% of patients and 28.73% reported currently engaging in NSSI (within at least the past few months). Analyses showed that those with a lifetime presence of NSSI had significantly greater general psychopathology, lower self-esteem, had suffered more transphobia, and experienced greater interpersonal problems than those without NSSI. Findings were similar when comparing current with non-current NSSI. Overall, natal male patients reported less social support than natal female patients, but current NSSI was more common in natal female patients. Regression analyses confirmed that natal female gender and greater general psychopathology predicted current and lifetime NSSI. Further analyses confirmed that general psychopathology itself could be predicted by transphobic experiences, low self-esteem, and interpersonal problems, but not by the use of cross-sex hormones."
- Non-suicidal self-injury in trans people: associations with psychological symptoms, victimization, interpersonal functioning, and perceived social support
    - "The sample consisted of 66.5% trans women and 33.5% trans men and 36.8% of them had a history of engaging in NSSI. The prevalence of NSSI was significantly higher in trans men (57.7%) compared with trans women (26.2%). Trans individuals with NSSI reported more psychological and interpersonal problems and perceived less social support compared with trans individuals without NSSI. Moreover, the probability of having experienced physical harassment related to being trans was highest in trans

women with NSSI (compared with those without NSSI). The study found that with respect to psychological symptoms, trans women reported significantly more intrapersonal and interpersonal symptoms compared with trans men. Finally, the results of the regression analysis showed that the probability of engaging in NSSI by trans individuals was significantly positively related to a younger age, being trans male, and reporting more psychological symptoms."

- Risk Factors for Eating Disorder Psychopathology within the Treatment Seeking Transgender Population: The Role of Cross-Sex Hormone Treatment.
    - "Many transgender people experience high levels of body dissatisfaction, which is one of the numerous factors known to increase vulnerability to eating disorder symptoms in the cisgender (non-trans) population. Cross-sex hormones can alleviate body dissatisfaction so might also alleviate eating disorder symptoms. This study aimed to explore risk factors for eating disorder symptoms in transgender people and the role of cross-sex hormones. Individuals assessed at a national transgender health service were invited to participate (N = 563). Transgender people not on cross-sex hormones reported higher levels of eating disorder psychopathology than people who were. High body dissatisfaction, perfectionism, anxiety symptoms, and low self-esteem were risk factors for eating psychopathology, but, after controlling for these, significant differences in eating psychopathology between people who were and were not on cross-sex hormones disappeared. Cross-sex hormones may alleviate eating disorder psychopathology. Given the high prevalence of transgender identities, clinicians at eating disorder services should assess for gender identity issues."

Long-Term Follow-Ups and Assessments

- Transsexualism—General outcome and prognostic factors: A five-year follow-up study of nineteen transsexuals in the process of changing sex
    - "Nineteen transsexuals, approved for sex reassignment, were followed-up after 5 years. Outcome was evaluated as changes in seven areas of social, psychological, and psychiatric functioning. At baseline the patients were evaluated according to axis I, II, V (DSM-III-R), SCID screen, SASB (Structural Analysis of Social Behavior), and DMT (Defense Mechanism Test). At follow-up all but 1 were treated with contrary sex hormones, 12 had completed sex reassignment surgery, and 3 females were waiting for phalloplasty. One male transsexual regretted the decision to change sex and had quit the process. Two transsexuals had still not had any surgery due to older age or ambivalence. Overall, 68% (n=13) had improved in at least two areas of functioning. In 3 cases (16%) outcome were judged as unsatisfactory and one of those regarded sex change as a failure. Another 3 patients were mainly unchanged after 5 years. Female transsexuals had a slightly better outcome, especially concerning establishing and maintaining partnerships and improvement in socioeconomic status compared to male transsexuals. Baseline factors associated with negative outcome (unchanged or worsened) were presence of a personality disorder and high number of fulfilled axis II criteria. SCID screen assessments had high prognostic power. Negative self-image, according to SASB, predicted a negative outcome, whereas DMT variables were not correlated to outcome."
- Long-term follow-up: psychosocial outcome of Belgian transsexuals after sex reassignment surgery

- "On the GAF (DSM-IV) scale the female-to-male transsexuals scored significantly higher than the male-to-females (85.2 versus 76.2). While no difference in psychological functioning (SCL-90) was observed between the study group and a normal population, subjects with a pre-existing psychopathology were found to have retained more psychological symptoms. **The subjects proclaimed an overall positive change in their family and social life. None of them showed any regrets about the SRS.** A homosexual orientation, a younger age when applying for SRS, and an attractive physical appearance were positive prognostic factors."

- Long Term Follow up After Sex Reassignment Surgery
  - "A long term follow up of 136 patients operated on for sex reassignment was done to evaluate the surgical outcome. Social and psychological adjustments were also investigated by a questionnaire in 90 of these 136 patients. Optimal results of the operation are essential for a successful outcome. **Personal and social instability before operation, unsuitable body build, and age over 30 years at operation correlated with unsatisfactory results. Adequate family and social support is important for postoperative functioning.** Sex reassignment had no influence on the person's ability to work."
- A Five-Year Follow-Up Study of Swedish Adults with Gender Identity Disorder
  - "This follow-up study evaluated the outcome of sex reassignment as viewed by both clinicians and patients, with an additional focus on the outcome based on sex and subgroups. Of a total of 60 patients approved for sex reassignment, 42 (25 male-to-female [MF] and 17 female-to-male [FM]) transsexuals completed a follow-up assessment after 5 or more years in the process or 2 or more years after completed sex reassignment surgery. Twenty-six (62%) patients had an early onset and 16 (38%) patients had a late onset; 29 (69%) patients had a homosexual sexual orientation and 13 (31%) patients had a non-homosexual sexual orientation (relative to biological sex). At index and follow-up, a semi-structured interview was conducted. At follow-up, 32 patients had completed sex reassignment surgery, five were still in process, and five—following their own decision—had abstained from genital surgery. No one regretted their reassignment. The clinicians rated the global outcome as favorable in 62% of the cases, compared to 95% according to the patients themselves, with no differences between the subgroups. Based on the follow-up interview, more than 90% were stable or improved as regards work situation, partner relations, and sex life, but 5–15% were dissatisfied with the hormonal treatment, results of surgery, total sex reassignment procedure, or their present general health. Most outcome measures were rated positive and substantially equal for MF and FM. Late-onset transsexuals differed from those with early onset in some respects: these were mainly MF (88 vs. 42%), older when applying for sex reassignment (42 vs. 28 years), and non-homosexually oriented (56 vs. 15%). In conclusion, **almost all patients were satisfied with the sex reassignment; 86% were assessed by clinicians at follow-up as stable or improved in global functioning.**"
- Follow-up of sex reassignment surgery in transsexuals: a Brazilian cohort
  - "This study examined the impact of sex reassignment surgery on the satisfaction with sexual experience, partnerships, and relationship with family members in a cohort of Brazilian transsexual patients. A group of 19 patients who received sex reassignment between 2000 and 2004 (18 male-to-female, 1 female-to-male) after a two-year evaluation by a multidisciplinary team, and who agreed to participate in the study,

completed a written questionnaire. Mean age at entry into the program was 31.21+/-8.57 years and mean schooling was 9.2+/-1.4 years. **None of the patients reported regret for having undergone the surgery.** Sexual experience was considered to have improved by 83.3% of the patients, and became more frequent for 64.7% of the patients. For 83.3% of the patients, sex was considered to be pleasurable with the neovagina/neopenis. In addition, 64.7% reported that initiating and maintaining a relationship had become easier. The number of patients with a partner increased from 52.6% to 73.7%. Family relationships improved in 26.3% of the cases, whereas 73.7% of the patients did not report a difference. None of the patients reported worse relationships with family members after sex reassignment. **In conclusion, the overall impact of sex reassignment surgery on this cohort of patients was positive.**"

- Regrets After Sex Reassignment Surgery: Journal of Psychology & Human Sexuality: Vol 5, No 4
    - "Using data draw from the follow-up literature covering the last 30 years, and the author's clinical data on 295 men and women after SRS, an estimation of the number of patients who regretted the operations is made. **Among female-to-male transsexuals after SRS, i.e., in men, no regrets were reported in the author's sample, and in the literature they amount to less than 1%. Among male-to-female transsexuals after SRS, i.e., in women, regrets are reported in 1-1.5%**. Poor differential diagnosis, failure to carry out the real-life- test, and poor surgical results seem to be the main reasons behind the regrets reported in the literature. According to three cases observed by the author in addition to personality traits the lack of proper care in treating the patients played a major role."
- Transsexualism: Treatment Outcome of Compliant and Noncompliant Patients
    - "The objective of the study was a follow-up of the treatment outcome of Finnish transsexuals who sought sex reassignment during the period 1970–2002 and a comparison of the results and duration of treatment of compliant and noncompliant patients. Fifteen male-to-female transsexuals and 17 female-to-male transsexuals who had undergone hormone and surgical treatment and legal sex reassignment in Finland completed a questionnaire on psychosocial data and on their experience with the different phases of clinical assessment and treatment. The changes in their vocational functioning and social and psychic adjustment were used as outcome indicators. The results and duration of the treatment of compliant and noncompliant patients were compared. **The patients benefited significantly from treatment.** The noncompliant patients achieved equally good results as the compliant ones, and did so in a shorter time. A good treatment outcome could be achieved even when the patient had told the assessing psychiatrist a falsified story of his life and sought hormone therapy, genital surgery, or legal sex reassignment on his own initiative without a recommendation from the psychiatrist. Based on these findings, it is recommended that the doctor-patient relationship be reconsidered and founded on frank cooperation."
- Long-Term Follow-Up of Adults with Gender Identity Disorder
    - "The aim of this study was to re-examine individuals with gender identity disorder after as long a period of time as possible. To meet the inclusion criterion, the legal recognition of participants' gender change via a legal name change had to date back at least 10 years. The sample comprised 71 participants (35 MtF and 36 FtM). The follow-up period was 10–24 years with a mean of 13.8 years (*SD* = 2.78). Instruments included a combination of qualitative and quantitative methods: Clinical interviews were conducted with the

participants, and they completed a follow-up questionnaire as well as several standardized questionnaires they had already filled in when they first made contact with the clinic. Positive and desired changes were determined by all of the instruments: Participants reported high degrees of well-being and a good social integration. Very few participants were unemployed, most of them had a steady relationship, and they were also satisfied with their relationships with family and friends. **Their overall evaluation of the treatment process for sex reassignment and its effectiveness in reducing gender dysphoria was positive. Regarding the results of the standardized questionnaires, participants showed significantly fewer psychological problems and interpersonal difficulties as well as a strongly increased life satisfaction at follow-up than at the time of the initial consultation.** Despite these positive results, the treatment of transsexualism is far from being perfect."

- Effects of Medical Interventions on Gender Dysphoria and Body Image: A Follow-Up Study
    - "At follow-up, 29 participants (14%) did not receive medical interventions, 36 hormones only (18%), and 136 hormones and surgery (68%). Most transwomen had undergone genital surgery, and most transmen chest surgery. **Overall, the levels of gender dysphoria and body dissatisfaction were significantly lower at follow-up compared with clinical entry.** Satisfaction with therapy responsive and unresponsive body characteristics both improved. High dissatisfaction at admission and lower psychological functioning at follow-up were associated with persistent body dissatisfaction."
- Surgical Satisfaction, Quality of Life, and Their Association After Gender-Affirming Surgery: A Follow-up Study
    - "We assessed the outcomes of gender-affirming surgery (GAS, or sex-reassignment surgery) 4 to 6 years after first clinical contact, and the associations between postoperative (dis)satisfaction and quality of life (QoL). Our multicenter, cross-sectional follow-up study involved persons diagnosed with gender dysphoria (*DSM-IV-TR*) who applied for medical interventions from 2007 until 2009. Of 546 eligible persons, 201 (37%) responded, of whom 136 had undergone GAS (genital, chest, facial, vocal cord and/or thyroid cartilage surgery). Main outcome measures were procedure performed, self-reported complications, and satisfaction with surgical outcomes (standardized questionnaires), QoL (Satisfaction With Life Scale, Subjective Happiness Scale, Cantril Ladder), gender dysphoria (Utrecht Gender Dysphoria Scale), and psychological symptoms (Symptom Checklist-90). **Postoperative satisfaction was 94% to 100%, depending on the type of surgery performed. Eight (6%) of the participants reported dissatisfaction and/or regret, which was associated with preoperative psychological symptoms or self-reported surgical complications (*OR* = 6.07)**. Satisfied respondents' QoL scores were similar to reference values; dissatisfied or regretful respondents' scores were lower. Therefore, dissatisfaction after GAS may be viewed as an indicator of unfavorable psychological and QoL outcomes."
- Transsexualism in Serbia: A Twenty-Year Follow-Up Study
    - "Applicants for sex reassignment in Serbia are relatively young. The sex ratio is close to 1:1. They often come from single-child families. More than 10% do not wish to undergo surgical sex reassignment. The prevalence of PCOS among FTM transsexuals was higher than in the general population but considerably lower than that reported in the literature

from other populations. **Of those who had undergone sex reassignment, none expressed regret for their decision.**"

- Long-term Assessment of the Physical, Mental, and Sexual Health among Transsexual Women
    - "**Compared with reference populations, transsexual women scored good on physical and mental level (SF-36). Gender-related bodily features were shown to be of high value. Appreciation of their appearance as perceived by others, as well as their own satisfaction with their self-image as women obtained a good score (8 and 9, respectively).** However, sexual functioning as assessed through FSFI was suboptimal when compared with biological women, especially the sublevels concerning arousal, lubrication, and pain. Superior scores concerning sexual function were obtained in those transsexual women who were in a relationship and in heterosexuals."
- Long-term follow-up of individuals undergoing sex reassignment surgery: Psychiatric morbidity and mortality
    - "*Background*: There is a lack of long-term register-based follow-up studies of sex-reassigned individuals concerning mortality and psychiatric morbidity. Accordingly, the present study investigated both mortality and psychiatric morbidity using a sample of individuals with transsexualism which comprised 98% ($n = 104$) of all individuals in Denmark. *Aims*: (1) To investigate psychiatric morbidity before and after sex reassignment surgery (SRS) among Danish individuals who underwent SRS during the period of 1978–2010. (2) To investigate mortality among Danish individuals who underwent SRS during the period of 1978–2010. *Method*: Psychiatric morbidity and mortality were identified by data from the Danish Psychiatric Central Research Register and the Cause of Death Register through a retrospective register study of 104 sex-reassigned individuals.

      Results: Overall, 27.9% of the sample were registered with psychiatric morbidity before SRS and 22.1% after SRS ($p =$ not significant). A total of 6.7% of the sample were registered with psychiatric morbidity both before and after SRS. **Significantly more psychiatric diagnoses were found before SRS for those assigned as female at birth. Ten individuals were registered as deceased post-SRS with an average age of death of 53.5 years.**

      Conclusions: **No significant difference in psychiatric morbidity or mortality was found between male to female and female to male (FtM) save for the total number of psychiatric diagnoses where FtM held a significantly higher number of psychiatric diagnoses overall.** Despite the over-representation of psychiatric diagnoses both pre- and post-SRS the study found that only a relatively limited number of individuals had received diagnoses both prior to and after SRS. This suggests that generally SRS may reduce psychological morbidity for some individuals while increasing it for others."
- The Reported Sex and Surgery Satisfactions of 28 Postoperative Male-to-Female Transsexual Patients
    - "From 1980 to July 1997 sixty-one male-to-femalegender transformation surgeries were performed at our university center by one author (A.M.). Data were collected from patients who had surgery up to 1994 (n = 47) to obtain a minimum follow-up of 3years; 28 patients were contacted. A mail questionnaire was supplemented by personal interviews with 11 patients and telephone interviews with remaining patients to obtain and clarify additional information. Physical and functional results of surgery were judged to be good,

with few patients requiring additional corrective surgery. General satisfaction was expressed over the quality of cosmetic (normal appearing genitalia) and functional (ability to perceive orgasm)results. Follow-up showed satisfied who believed they had normal appearing genitalia and the ability to experience orgasm. Most patients were able to return to their jobs and live a more satisfactory social and personal life. One significant outcome was the importance of proper preparation of patients for surgery and especially the need for additional postoperative psychotherapy. **None of the patients regretted having had surgery. However, somewhere, to a degree, disappointed because of difficulties experienced postoperatively in adjusting satisfactorily women both in their relationships with men and in living their lives generally as women.** Findings of this study make a strong case for making a change in the Harry Benjamin Standards of Care to include a period of postoperative psychotherapy."

Literature Reviews and/or Guidelines

Biased Disclaimer: Strong personal dislike of the next four sources. Displeased by the conflation of gender non-conforming with transsexuals that many of these sources do, due to fundamental differences in psychology, medical treatment, and societal role. However, a diversity of sources is vital for a well-rounded opinion, and the information contained within is still valuable in that regard.

- Guidelines for psychological practice with transgender and gender nonconforming people.
    - "In 2015, the American Psychological Association adopted *Guidelines for Psychological Practice with Transgender and Gender Nonconforming Clients* in order to describe affirmative psychological practice with transgender and gender nonconforming (TGNC) clients. There are 16 guidelines in this document that guide TGNC-affirmative psychological practice across the lifespan, from TGNC children to older adults. The Guidelines are organized into five clusters: (a) foundational knowledge and awareness; (b) stigma, discrimination, and barriers to care; (c) lifespan development; (d) assessment, therapy, and intervention; and (e) research, education, and training. In addition, the guidelines provide attention to TGNC people across a range of gender and racial/ethnic identities. The psychological practice guidelines also attend to issues of research and how psychologists may address the many social inequities TGNC people experience. (APA PsycInfo Database Record (c) 2019 APA, all rights reserved)"
- Adult development and quality of life of transgender and gender nonconforming people
    - "Pervasive stigma and discrimination attached to gender nonconformity affect the health of transgender people across the lifespan, particularly when it comes to mental health and well-being. Despite the related challenges, transgender and gender nonconforming people may develop resilience over time. Social support and affirmation of gender identity play herein a critical role. Although there is a growing awareness of diversity in gender identity and expression among this population, a comprehensive understanding of biopsychosocial development beyond the gender binary and beyond transition is lacking."
- Report of the American Psychiatric Association Task Force on Treatment of Gender Identity Disorder

    - "Both the diagnosis and treatment of Gender Identity Disorder (GID) are controversial. Although linked, they are separate issues and the DSM does not evaluate treatments. The Board of Trustees (BOT) of the American Psychiatric Association (APA), therefore, formed a Task Force charged to perform a critical review of the literature on the treatment of GID at different ages, to assess the quality of evidence pertaining to treatment, and to prepare a report that included an opinion as to whether or not sufficient credible literature exists for development of treatment recommendations by the APA. The literature on treatment of gender dysphoria in individuals with disorders of sex development was also assessed. The completed report was accepted by the BOT on September 11, 2011. The quality of evidence pertaining to most aspects of treatment in all subgroups was determined to be low; however, areas of broad clinical consensus were identified and were deemed sufficient to support recommendations for treatment in all subgroups. With subjective improvement as the primary outcome measure, current evidence was judged sufficient to support recommendations for adults in the form of an evidence-based APA Practice Guideline with gaps in the empirical data supplemented by clinical consensus. The report recommends that the APA take steps beyond drafting treatment recommendations. These include issuing position statements to clarify the APA's position regarding the medical necessity of treatments for GID, the ethical bounds of treatments of gender variant minors, and the rights of persons of any age who are gender variant, transgender or transsexual."
- Outcomes of Treatment for Gender Dysphoria: Journal of Sex Education and Therapy: Vol 24, No 3
    - "This paper reviews the empirical research on the psychosocial outcomes of treatment for gender dysphoria. Recent research has highlighted the heterogeneity of transgendered experiences. There are four possible outcomes for patients who present with the dilemma of gender dysphoria: an unresolved outcome, acceptance of one's given gender, engaging in a cross-gender role on a part-time basis, and making a full-time transition to the other gender role. Clinical work, but not empirical research, suggests that some individuals with gender dysphoria may come to accept their given gender role through psychological treatment. Many individuals find that it is psychologically sufficient to express the transgendered part of themselves through such activities as cross-dressing or gender blending. The large body of research on the outcome of gender reassignment surgery indicates that, for the majority of those who undergo this process, the outcome is positive. Predictors of a good outcome include good pre-reassignment psychological adjustment, family support, at least 1 year of living in the desired role, consistent use of hormones, psychological treatment, and good surgical outcomes. The outcome literature provides strong support for adherence to the Standards of Care of the Harry Benjamin International Gender Dysphoria Association. Implications to be drawn from this research include an appreciation of the diversity of transgendered experience, the need for more research on non-reassignment resolutions to gender dysphoria, and the importance of assisting the transgendered individual to identify the resolution that best suits him or her."

World Medical Authorities on Transsexualism

- Resolution on Transgender, Gender Identity, and Gender Expression Non-Discrimination

    - "THEREFORE BE IT FURTHER RESOLVED that APA recognizes the efficacy, benefit and medical necessity of gender transition treatments for appropriately evaluated individuals and calls upon public and private insurers to cover these medically necessary treatments;"
- AMERICAN MEDICAL ASSOCIATION HOUSE OF DELEGATES Resolution: 122 (A-08) Introduced by: Resident and Fellow Section, Massachusett
    - "Whereas, The World Professional Association For Transgender Health, Inc. ("WPATH") is the leading international, interdisciplinary professional organization devoted to the understanding and treatment of gender identity disorders, and has established internationally accepted Standards of Care for providing medical treatment for people with GID, including mental health care, hormone therapy and sex reassignment surgery, which are designed to promote the health and welfare of persons with GID and are recognized within the medical community to be the standard of care for treating people with GID;"
- Lesbian, Gay, Bisexual, and Transgender Health Disparities: Executive Summary of a Policy Position Paper From the American College of Physicians | Annals of Internal Medicine
    - "Research shows that when transgender persons receive individual, medically appropriate care, they have improved mental health, reduction in suicide rates, and lower health care costs overall because of fewer mental health–related and substance abuse–related costs … Many professional medical organizations, including the American Medical Association, American Psychological Association, American Psychiatric Association, American Congress of Obstetricians and Gynecologists, and American Academy of Family Physicians, consider gender transition–related medical services medically necessary."
- American Academy of Pediatrics: Supporting and Caring for Transgender Children
    - "RESOLVED, That the American Academy of Family Physicians (AAFP) support efforts to require insurers to provide coverage for comprehensive care of transgendered individuals including medical care, screening tests based on medical need rather than gender, mental health care, and, when medically necessary, gender reassignment surgery."
- American Academy of Pediatrics: Supporting and Caring for Transgender Children
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- National Association of Social Workers
    - Link now leads to an Error page. Access may still be possible through other means.
- Supporting transgender and gender-diverse people
    - "A review of academic publications has recently been completed and submitted for publication (Wright et al., 2018). The findings suggest that specific treatments to persuade transgender and gender-diverse people to accept their gender as assigned at birth are rare, but there is evidence of barriers to transgender people receiving appropriate help to enable medical and social transition. Denying access to gender-affirming treatment is likely to have a detrimental effect on the wellbeing of transgender and gender-diverse people"
- Gender dysphoria - guide for GPs and other Health Care Staff - Final 24.4.13(1).docx

    - "*Transsexualism* is an extreme form of Gender Dysphoria. It is the desire to transition and be accepted as a member of a sex other than that assigned at birth, and to make one's body as congruent as possible, typically through hormones (endocrine treatment) and surgery. Transsexualism is coded in the current International Classification of Diseases as ICD-10 F64.0."
- Gender dysphoria - Treatment

Sexual Dimorphism of Brains

The subject of the sources largely include one or more of the following:

Genetics, Gender Identity, Sexual Orientation, Hormones, Hormonal Imbalances and Sex Disorders, Gendered Social Development and Socialization, Sexual Dimorphism, Cognitive Development, Fertility

Studies of Cissexuals

- Normal Sexual Dimorphism of the Adult Human Brain Assessed by In Vivo Magnetic Resonance Imaging
    - "The etiology and consistency of findings on normal sexual dimorphisms of the adult human brain are unresolved. In this study, we present a comprehensive evaluation of normal sexual dimorphisms of cortical and subcortical brain regions, using *in vivo* magnetic resonance imaging, in a community sample of 48 normal adults. The men and women were similar in age, education, ethnicity, socioeconomic status, general intelligence and handedness. Forty-five brain regions were assessed based on T_1-weighted three-dimensional images acquired from a 1.5 T magnet. Sexual dimorphisms of adult brain volumes were more evident in the cortex, with women having larger volumes, relative to cerebrum size, particularly in frontal and medial paralimbic cortices. Men had larger volumes, relative to cerebrum size, in frontomedial cortex, the amygdala and hypothalamus. A permutation test showed that, compared to other brain areas assessed in this study, there was greater sexual dimorphism among brain areas that are homologous with those identified in animal studies showing greater levels of sex steroid receptors during critical periods of brain development. These findings have implications for developmental studies that would directly test hypotheses about mechanisms relating sex steroid hormones to sexual dimorphisms in humans."
- Sexual Differentiation of the Bed Nucleus of the Stria Terminalis in Humans May Extend into Adulthood
    - "Gonadal steroids have remarkable developmental effects on sex-dependent brain organization and behavior in animals. Presumably, fetal or neonatal gonadal steroids are also responsible for sexual differentiation of the human brain. A limbic structure of

special interest in this regard is the sexually dimorphic central subdivision of the bed nucleus of the stria terminalis (BSTc), because its size has been related to the gender identity disorder transsexuality. To determine at what age the BSTc becomes sexually dimorphic, the BSTc volume in males and females was studied from midgestation into adulthood. Using vasoactive intestinal polypeptide and somatostatin immunocytochemical staining as markers, we found that the BSTc was larger and contains more neurons in men than in women. However, this difference became significant only in adulthood, showing that sexual differentiation of the human brain may extend into the adulthood. The unexpectedly late sexual differentiation of the BSTc is discussed in relation to sex differences in developmental, adolescent, and adult gonadal steroid levels."

- Is there a gender difference of somatostatin-receptor density in the human brain?
    - "Animal experiments and observations in human brains have convincingly shown that sexual differentiation not only concerns the genitalia but also the brain. This has been investigated also in the light of a possible explanation of a presumed biological aetiology of transsexuality. The volume of the central subdivision of the bed nucleus of the stria terminalis, a brain area that is essential for sexual behaviour, has been reported to be larger in men than in women. Additionally, the number of somatostatin expressing neurons in this region was shown to be higher in men than in women. As neuronal production of somatostatin is involved the idea is striking whether somatostatin-receptor density in the cortex of cerebral hemispheres might be related to gender identity. We investigated in vivo the density of somatostatin-receptors in selected regions of the human brain in both sexes by means of receptor scintigraphy. Basal ganglia tracer uptake of 111-In-Pentreotide was equally low in both genders at 0,80% +/ 0,26 (related to tracer uptake of the whole brain layer). Temporal cortex accumulated at 2,9% +/ 1,1 in men and at 2,3% +/ 0,76 in women. Frontal brain region had an uptake of 3,0% +/ 1,4 in male and of 2,5% +/ 1,3 in female. This shows a tendency in males for relatively augmented uptake indicating higher somatostatin receptor density in temporal and frontal cerebral cortex."
- The role of the androgen receptor in CNS masculinization
    - "The medial posterior region of the bed nucleus of the stria terminalis (BSTMP) and the locus coeruleus (LC) show opposite patterns of sexual dimorphism. The BSTMP in males is greater in volume and number of neurons than in females (male > female) while in the LC, the opposite is true (female > male). To investigate the possible role of the androgen receptor (AR) in the masculinization of these two structures, males with the testicular feminization mutation (Tfm) were compared to their control littermate males. No differences were seen in the number of neurons of the BSTMP between Tfm and their control littermate males, while in the LC, Tfm males have a greater number of neurons than their control littermate males. These results show that the AR is involved in the control of neuron number in the LC but not in the BSTMP. Results based on the LC suggest that when females have a larger brain area than males, masculinization in males may be achieved through the AR, with androgens perhaps decreasing cell survival."
- Sex differences in the human olfactory system
    - "The olfactory system (accessory) implicated in reproductive physiology and behavior in mammals is sexually dimorphic. These brain sex differences present two main characteristics: they are seen in neural circuits related to sexual behavior and sexual

physiology and they take one of two opposite morphological patterns (male>female or female>male). The present work reports sex differences in the olfactory system in a large homogeneous sample of men (40) and women (51) using of voxel-based morphology. Gray matter concentration showed sexual dimorphism in several olfactory regions. Women have a higher concentration in the orbitofrontal cortex involving Brodmann's areas 10, 11 and 25 and temporomedial cortex (bilateral hippocampus and right amygdala), as well as their left basal insular cortex. In contrast, men show a higher gray matter concentration in the left entorhinal cortex (Brodmann's area 28), right ventral pallidum, dorsal left insular cortex and a region of the orbitofrontal cortex (Brodmann's area 25). This study supports the hypothesis that the mammalian olfactory system is a sexually dimorphic network and provides a theoretical framework for the morphofunctional approach to sex differences in the human brain."

- Gender Differences in Emotion Regulation: An fMRI Study of Cognitive Reappraisal
    - "Despite strong popular conceptions of gender differences in emotionality and striking gender differences in the prevalence of disorders thought to involve emotion dysregulation, the literature on the neural bases of emotion regulation is nearly silent regarding gender differences (Gross, 2007; Ochsner & Gross, in press). The purpose of the present study was to address this gap in the literature. Using functional magnetic resonance imaging, we asked male and female participants to use a cognitive emotion regulation strategy (reappraisal) to down-regulate their emotional responses to negatively valenced pictures. Behaviorally, men and women evidenced comparable decreases in negative emotion experience. Neurally, however, gender differences emerged. Compared with women, men showed (a) lesser increases in prefrontal regions that are associated with reappraisal, (b) greater decreases in the amygdala, which is associated with emotional responding, and (c) lesser engagement of ventral striatal regions, which are associated with reward processing. We consider two non-competing explanations for these differences. First, men may expend less effort when using cognitive regulation, perhaps due to greater use of automatic emotion regulation. Second, women may use positive emotions in the service of reappraising negative emotions to a greater degree. We then consider the implications of gender differences in emotion regulation for understanding gender differences in emotional processing in general, and gender differences in affective disorders."
- The Genetics of Sex Differences in Brain and Behavior
    - "Biological differences between men and women contribute to many sex-specific illnesses and disorders. Historically, it was argued that such differences were largely, if not exclusively, due to gonadal hormone secretions. However, emerging research has shown that some differences are mediated by mechanisms other than the action of these hormone secretions and in particular by products of genes located on the X and Y chromosomes, which we refer to as direct genetic effects. This paper reviews the evidence for direct genetic effects in behavioral and brain sex differences. We highlight the `four core genotypes' model and sex differences in the midbrain dopaminergic system, specifically focusing on the role of *Sry*. We also discuss novel research being done on unique populations including people attracted to the same sex and people with a cross-gender identity. As science continues to advance our understanding of biological sex differences, a new field is emerging that is aimed at better addressing the needs of

both sexes: gender-based biology and medicine. Ultimately, the study of the biological basis for sex differences will improve healthcare for both men and women."

- Sexual Differentiation of the Human Brain and Male/Female Behaviour
    - "Once the differentiation of our sexual organs into male or female is settled, the next thing to be differentiated is the brain. The difference in brain structures resulting from the interaction of sex hormones and developing brain cells, is thought to be the basis of sex differences in behaviour, in gender identity, in gender roles, in our sexual orientation (hetero-, bi- or homosexuality) and in the obvious sex differences in cognition and aggressive behaviour. Our sexual orientation is determined during early foetal development, under the influence of our genetic background and of factors that affect the complex interactions between sex hormones and the developing brain. Although it has often been postulated that postnatal development is also important for the direction of our sexual differentiation, any solid proof for this is lacking. The broadly accepted view on the importance of the social environment on sexual differentiation has been extensively put into words by Simone de Beauvoir and others. It turns out, however, that sex differences revealed through play, drawings and aggression are determined by exposure to hormones in the womb rather than by what society demands later on. The apparent impossibility to get someone to change their sexual orientation is a major argument against the importance of the social environment in the emergence of homosexuality, as well as against the idea that homosexuality is a lifestyle choice. Our sexual orientation is fixed during prenatal development and is beyond influencing in adulthood. Apparently, and despite the feminist ideals, we tend to choose what best fits our programmed (by natural sexual selection developed) brains. Our sexually differential brains will not lend themselves for a completely equal division of tasks between men and women in the family or on the labour market. There is great public interest in research of the brain and in research of our sexual behaviour, but the combination of these two subjects has turned out to be dynamite."
- Sex-related variation in human behavior and the brain
    - "Male and female fetuses differ in testosterone concentrations beginning as early as week 8 of gestation. This early hormone difference exerts permanent influences on brain development and behavior. Contemporary research shows that hormones are particularly important for the development of sex-typical childhood behavior, including toy choices, which until recently were thought to result solely from sociocultural influences. Prenatal testosterone exposure also appears to influence sexual orientation and gender identity, as well as some, but not all, sex-related cognitive, motor and personality characteristics. Neural mechanisms responsible for these hormone-induced behavioral outcomes are beginning to be identified, and current evidence suggests involvement of the hypothalamus and amygdala, as well as interhemispheric connectivity, and cortical areas involved in visual processing."
- Gender Differences in White Matter Microstructure
    - *"Results:* Men had higher fractional anisotropy (FA) in cerebellar white matter and in the left superior longitudinal fasciculus; women had higher FA in the corpus callosum, confirmed by ROI.

      Discussion: The size of the differences was substantial - of the same order as that attributed to some pathology – suggesting gender may be a potentially significant confound in unbalanced clinical studies. There are several previous reports of difference

in the corpus callosum, though they disagree on the direction of difference; our findings in the cerebellum and the superior longitudinal fasciculus have not previously been noted. The higher FA in women may reflect greater efficiency of a smaller corpus callosum. The relatively increased superior longitudinal fasciculus and cerebellar FA in men may reflect their increased language lateralisation and enhanced motor development, respectively."

- Sex differences in the structural connectome of the human brain
    - "Sex differences in human behavior show adaptive complementarity: Males have better motor and spatial abilities, whereas females have superior memory and social cognition skills. Studies also show sex differences in human brains but do not explain this complementarity. In this work, we modeled the structural connectome using diffusion tensor imaging in a sample of 949 youths (aged 8–22 y, 428 males and 521 females) and discovered unique sex differences in brain connectivity during the course of development. Connection-wise statistical analysis, as well as analysis of regional and global network measures, presented a comprehensive description of network characteristics. In all supratentorial regions, males had greater within-hemispheric connectivity, as well as enhanced modularity and transitivity, whereas between-hemispheric connectivity and cross-module participation predominated in females. However, this effect was reversed in the cerebellar connections. Analysis of these changes developmentally demonstrated differences in trajectory between males and females mainly in adolescence and in adulthood. Overall, the results suggest that male brains are structured to facilitate connectivity between perception and coordinated action, whereas female brains are designed to facilitate communication between analytical and intuitive processing modes."
- Sex differences in the human brain and the impact of sex chromosomes and sex hormones
    - "While there has been increasing support for the existence of cerebral sex differences, the mechanisms underlying these differences are unclear. Based on animal data, it has long been believed that sexual differentiation of the brain is primarily linked to organizational effects of fetal testosterone. This view is, however, in question as more recent data show the presence of sex differences before the onset of testosterone production. The present study focuses on the impact that sex chromosomes might have on these differences. Utilizing the inherent differences in sex and X-chromosome dosage among XXY males, XY males, and XX females, comparative voxel-based morphometry was conducted using sex hormones and sex chromosomes as covariates. Sex differences in the cerebellar and precentral gray matter volumes (GMV) were found to be related to X-chromosome dosage, whereas sex differences in the amygdala, the parahippocamus, and the occipital cortex were linked to testosterone levels. An increased number of sex chromosomes was associated with reduced GMV in the amygdala, caudate, and the temporal and insular cortices, with increased parietal GMV and reduced frontotemporal white matter volume. No selective, testosterone independent, effect of the Y-chromosome was detected. Based on these observations, it was hypothesized that programming of the motor cortex and parts of cerebellum is mediated by processes linked to X-escapee genes, which do not have Y-chromosome homologs, and that programming of certain limbic structures involves testosterone and X-chromosome escapee genes with Y-homologs."

- Sex differences in cortical thickness and their possible genetic and sex hormonal underpinnings
    - "Although it has been shown that cortical thickness (Cth) differs between sexes, the underlying mechanisms are unknown. Seeing as XXY males have 1 extra X chromosome, we investigated the possible effects of X- and sex-chromosome dosage on Cth by comparing data from 31 XXY males with 39 XY and 47 XX controls. Plasma testosterone and estrogen were also measured in an effort to differentiate between possible sex-hormone and sex-chromosome gene effects. Cth was calculated with FreeSurfer software. Parietal and occipital Cth was greater in XX females than XY males. In these regions Cth was inversely correlated with z-normalized testosterone. In the motor strip, the cortex was thinner in XY males compared with both XX females and XXY males, indicating the possibility of an X-chromosome gene-dosage effect. XXY males had thinner right superior temporal and left middle temporal cortex, and a thicker right orbitofrontal cortex and lingual cortex than both control groups. Based on these data and previous reports from women with XO monosomy, it is hypothesized that programming of the motor cortex is influenced by processes linked to X-escapee genes, which do not have Y-chromosome homologs, and that programming of the superior temporal cortex is mediated by X-chromosome escapee genes with Y-homologs."
- Impact of sex and gonadal steroids on neonatal brain structure
    - "There are numerous reports of sexual dimorphism in brain structure in children and adults, but data on sex differences in infancy are extremely limited. Our primary goal was to identify sex differences in neonatal brain structure. Our secondary goal was to explore whether brain structure was related to androgen exposure or sensitivity. Two hundred and ninety-three neonates (149 males) received high-resolution structural magnetic resonance imaging scans. Sensitivity to androgen was measured using the number of cytosine, adenine, guanine (CAG) triplets in the androgen receptor gene and the ratio of the second to fourth digit, provided a proxy measure of prenatal androgen exposure. There was a significant sex difference in intracranial volume of 5.87%, which was not related to CAG triplets or digit ratios. Tensor-based morphometry identified extensive areas of local sexual dimorphism. Males had larger volumes in medial temporal cortex and rolandic operculum, and females had larger volumes in dorsolateral prefrontal, motor, and visual cortices. Androgen exposure and sensitivity had minor sex-specific effects on local gray matter volume, but did not appear to be the primary determinant of sexual dimorphism at this age. Comparing our study with the existing literature suggests that sex differences in cortical structure vary in a complex and highly dynamic way across the human lifespan."
- Sexual Dimorphism in the Human Olfactory Bulb: Females Have More Neurons and Glial Cells than Males
    - "Sex differences in the human olfactory function reportedly exist for olfactory sensitivity, odorant identification and memory, and tasks in which odors are rated based on psychological features such as familiarity, intensity, pleasantness, and others. Which might be the neural bases for these behavioral differences? The number of cells in olfactory regions, and especially the number of neurons, may represent a more accurate indicator of the neural machinery than volume or weight, but besides gross volume measures of the human olfactory bulb, no systematic study of sex differences in the absolute number of cells has yet been undertaken. In this work, we investigate a possible

sexual dimorphism in the olfactory bulb, by quantifying postmortem material from 7 men and 11 women (ages 55–94 years) with the isotropic fractionator, an unbiased and accurate method to estimate absolute cell numbers in brain regions. Female bulbs weighed 0.132 g in average, while male bulbs weighed 0.137 g, a non-significant difference; however, the total number of cells was 16.2 million in females, and 9.2 million in males, a significant difference of 43.2%. The number of neurons in females reached 6.9 million, being no more than 3.5 million in males, a difference of 49.3%. The number of non-neuronal cells also proved higher in women than in men: 9.3 million and 5.7 million, respectively, a significant difference of 38.7%. The same differences remained when corrected for mass. Results demonstrate a sex-related difference in the absolute number of total, neuronal and non-neuronal cells, favoring women by 40–50%. It is conceivable that these differences in quantitative cellularity may have functional impact, albeit difficult to infer how exactly this would be, without knowing the specific circuits cells make. However, the reported advantage of women as compared to men may stimulate future work on sex dimorphism of synaptic microcircuitry in the olfactory bulb."

- Sexual dimorphism in ALS: exploring gender-specific neuroimaging signatures
    - "Our objective was to explore neuroanatomical differences between female and male ALS patients in the context of sexual dimorphism in healthy controls. Fourteen female ALS patients, 13 male ALS patients, 22 healthy male controls and 20 healthy female controls were recruited into a comprehensive neuroimaging study. Cortical thickness measurements and diffusion tensor imaging (DTI) were utilized to explore gender-specific anatomical vulnerability. DTI analysis across all study groups revealed higher fractional anisotropy in association with male gender in the brainstem, cerebellum, fornix, thalamus, anterior forceps and corticospinal tracts accounting for diagnosis and age. While females showed a trend of higher age-adjusted cortical thickness in the right parieto-occipital and left mid-frontal regions, males demonstrated higher cortical thickness in the left lingual and left superior temporal regions, accounting for diagnosis. Significant multifocal white matter differences have also been identified between healthy male and female controls. In conclusion, sexual dimorphism is an overlooked and potentially confounding factor in admixed ALS neuroimaging studies. Our results suggest that gender is an additional dimension of disease heterogeneity in ALS. Given the significant pre- and post-morbid gender differences, we feel that ALS imaging studies should be controlled for gender or, alternatively, single gender studies should be considered."
- Gender Influence on White Matter Microstructure: A Tract-Based Spatial Statistics Analysis
    - "*Results:* Men had higher FA in the superior cerebellar peduncles and women had higher FA in corpus callosum in both the first and second samples. The higher SLF FA in men was not found in either sample.
      Discussion: We confirmed our previous, controversial finding of increased FA in the corpus callosum in women, and increased cerebellar FA in men. The corpus callosum FA difference offers some explanation for the otherwise puzzling advantage in inter-callosal transfer time shown in women; the cerebellar FA difference may be associated with the developmental motor advantage shown in men."
- Asymmetry within and around the human planum temporale is sexually dimorphic and influenced by genes involved in steroid hormone receptor activity

    - "The genetic determinants of cerebral asymmetries are unknown. Sex differences in asymmetry of the planum temporale (PT), that overlaps Wernicke's classical language area, have been inconsistently reported. Meta-analysis of previous studies has suggested that publication bias established this sex difference in the literature. Using probabilistic definitions of cortical regions we screened over the cerebral cortex for sexual dimorphisms of asymmetry in 2337 healthy subjects, and found the PT to show the strongest sex-linked asymmetry of all regions, which was supported by two further datasets, and also by analysis with the FreeSurfer package that performs automated parcellation of cerebral cortical regions. We performed a genome-wide association scan (GWAS) meta-analysis of PT asymmetry in a pooled sample of 3095 subjects, followed by a candidate-driven approach which measured a significant enrichment of association in genes of the 'steroid hormone receptor activity' and 'steroid metabolic process' pathways. Variants in the genes and pathways identified may affect the role of the PT in language cognition."
- Sex differences in effective fronto-limbic connectivity during negative emotion processing
    - "*Results:* Subjective ratings of negative emotional images were higher in women than in men. Across sexes, significant activations were observed in the dorso-medial prefrontal cortex (dmPFC) and the right amygdala. Granger connectivity from right amygdala was significantly greater than that from dmPFC during the 'high negative' condition, an effect driven by men. Magnitude of this effect correlated negatively with highly negative image ratings and feminine traits and positively with testosterone levels.
      Discussion: These results highlight critical sex differences in brain connectivity during negative emotion processing and point to the fact that both biological (sex steroid hormones) and psychosocial (gender role and identity) variables contribute to them. As the dmPFC is involved in social cognition and action planning, and the amygdala-in threat detection, the connectivity results suggest that compared to women, men have a more evaluative, rather than purely affective, brain response during negative emotion processing."
- Progressive Gender Differences of Structural Brain Networks in Healthy Adults: A Longitudinal, Diffusion Tensor Imaging Study
    - "Sexual dimorphism in the brain maturation during childhood and adolescence has been repeatedly documented, which may underlie the differences in behaviors and cognitive performance. However, our understanding of how gender modulates the development of structural connectome in healthy adults is still not entirely clear. Here we utilized graph theoretical analysis of longitudinal diffusion tensor imaging data over a five-year period to investigate the progressive gender differences of brain network topology. The brain networks of both genders showed prominent economical "small-world" architecture (high local clustering and short paths between nodes). Additional analysis revealed a more economical "small-world" architecture in females as well as a greater global efficiency in males regardless of scan time point. At the regional level, both increased and decreased efficiency were found across the cerebral cortex for both males and females, indicating a compensation mechanism of cortical network reorganization over time. Furthermore, we found that weighted clustering coefficient exhibited significant gender-time interactions, implying different development trends between males and females. Moreover, several specific brain regions (e.g., insula, superior temporal gyrus, cuneus, putamen, and

parahippocampal gyrus) exhibited different development trajectories between males and females. Our findings further prove the presence of sexual dimorphism in brain structures that may underlie gender differences in behavioral and cognitive functioning. The sex-specific progress trajectories in brain connectome revealed in this work provide an important foundation to delineate the gender related pathophysiological mechanisms in various neuropsychiatric disorders, which may potentially guide the development of sex-specific treatments for these devastating brain disorders."

- Measuring the effects of aging and sex on regional brain stiffness with MR elastography in healthy older adults
    - "In conclusion, our study confirms that in an older population, as the brain ages, there is softening; however, this is not true for all regions of the brain. In addition, stiffness effects due to sex exist in the occipital and temporal lobes. Although the mechanisms behind these changes are not fully understood, it is important that they are recognized, especially when comparing different control and patient cohorts."
- Marked effects of intracranial volume correction methods on sex differences in neuroanatomical structures: a HUNT MRI study
    - "To date, there is no consensus whether sexual dimorphism in the size of neuroanatomical structures exists, or if such differences are caused by choice of intracranial volume (ICV) correction method. When investigating volume differences in neuroanatomical structures, corrections for variation in ICV are used. Commonly applied methods are the ICV-proportions, ICV-residuals and ICV as a covariate of no interest, ANCOVA. However, these different methods give contradictory results with regard to presence of sex differences. Our aims were to investigate presence of sexual dimorphism in 18 neuroanatomical volumes unrelated to ICV-differences by using a large ICV-matched subsample of 304 men and women from the HUNT-MRI general population study, and further to demonstrate in the entire sample of 966 healthy subjects, which of the ICV-correction methods gave results similar to the ICV-matched subsample. In addition, sex-specific subsamples were created to investigate whether differences were an effect of head size or sex. Most sex differences were related to volume scaling with ICV, independent of sex. Sex differences were detected in a few structures; amygdala, cerebellar cortex, and 3rd ventricle were larger in men, but the effect sizes were small. The residuals and ANCOVA methods were most effective at removing the effects of ICV. The proportions method suffered from systematic errors due to lack of proportionality between ICV and neuroanatomical volumes, leading to systematic mis-assignment of structures as either larger or smaller than their actual size. Adding additional sexual dimorphic covariates to the ANCOVA gave opposite results of those obtained in the ICV-matched subsample or with the residuals method. The findings in the current study explain some of the considerable variation in the literature on sexual dimorphisms in neuroanatomical volumes. In conclusion, sex plays a minor role for neuroanatomical volume differences; most differences are related to ICV."
- Brain feminization requires active repression of masculinization via DNA methylation
    - "The developing mammalian brain is destined for a female phenotype unless exposed to gonadal hormones during a perinatal sensitive period. It has been assumed that the undifferentiated brain is masculinized by direct induction of transcription by ligand-activated nuclear steroid receptors. We found that a primary effect of gonadal steroids in the highly sexually dimorphic preoptic area (POA) is to reduce activity of DNA

methyltransferase (Dnmt) enzymes, thereby decreasing DNA methylation and releasing masculinizing genes from epigenetic repression. Pharmacological inhibition of Dnmts mimicked gonadal steroids, resulting in masculinized neuronal markers and male sexual behavior in female rats. Conditional knockout of the de novo Dnmt isoform, Dnmt3a, also masculinized sexual behavior in female mice. RNA sequencing revealed gene and isoform variants modulated by methylation that may underlie the divergent reproductive behaviors of males versus females. Our data show that brain feminization is maintained by the active suppression of masculinization via DNA methylation."

- Development of cortical shape in the human brain from 6 to 24months of age via a novel measure of shape complexity
    - "The quantification of local surface morphology in the human cortex is important for examining population differences as well as developmental changes in neurodegenerative or neurodevelopmental disorders. We propose a novel cortical shape measure, referred to as the 'shape complexity index' (SCI), that represents localized shape complexity as the difference between the observed distributions of local surface topology, as quantified by the shape index (SI) measure, to its best fitting simple topological model within a given neighborhood. We apply a relatively small, adaptive geodesic kernel to calculate the SCI. Due to the small size of the kernel, the proposed SCI measure captures fine differences of cortical shape. With this novel cortical feature, we aim to capture comparatively small local surface changes that capture a) the widening versus deepening of sulcal and gyral regions, as well as b) the emergence and development of secondary and tertiary sulci. Current cortical shape measures, such as the gyrification index (GI) or intrinsic curvature measures, investigate the cortical surface at a different scale and are less well suited to capture these particular cortical surface changes. In our experiments, the proposed SCI demonstrates higher complexity in the gyral/sulcal wall regions, lower complexity in wider gyral ridges and lowest complexity in wider sulcal fundus regions. In early postnatal brain development, our experiments show that SCI reveals a pattern of increased cortical shape complexity with age, as well as sexual dimorphisms in the insula, middle cingulate, parieto-occipital sulcal and Broca's regions. Overall, sex differences were greatest at 6months of age and were reduced at 24months, with the difference pattern switching from higher complexity in males at 6months to higher complexity in females at 24months. This is the first study of longitudinal, cortical complexity maturation and sex differences, in the early postnatal period from 6 to 24months of age with fine scale, cortical shape measures. These results provide information that complement previous studies of gyrification index in early brain development."
- A characterization of performance by men and women in a virtual Morris water task: a large and reliable sex difference
    - "In many mammalian species, it is known that males and females differ in place learning ability. The performance by men and women is commonly reported to also differ, despite a large amount of variability and ambiguity in measuring spatial abilities. In the non-human literature, the gold standard for measuring place learning ability in mammals is the Morris water task. This task requires subjects to use the spatial arrangement of cues outside of a circular pool to swim to a hidden goal platform located in a fixed location. We used a computerized version of the Morris water task to assess whether this task will generalize into the human domain and to examine whether sex differences exist

in this domain of topographical learning and memory. Across three separate experiments, varying in attempts to maximize spatial performance, we consistently found males navigate to the hidden platform better than females across a variety of measures. The effect sizes of these differences are some of the largest ever reported and are robust and replicable across experiments. These results are the first to demonstrate the effectiveness and utility of the virtual Morris water task for humans and show a robust sex difference in virtual place learning."

- [Sex differentiation of central nervous system--brain of man and woman]
    - "Sex differentiation of human brain is mostly dependent on the prenatal exposure to androgen(testosterone). Congenital aromatase deficiency does not disturb male brain development in men. This is quite different from experimental evidence from rodents whose brains need intraneuronal aromatization from androgen to estrogen to induce sex differentiation. There is evidence for male-female differences in brain structures. Some of them(INHA-3) appear to be related with sexual orientation. The other(BNST) might participate in forming gender-identity. In addition, sexually dimorphic features are recognized in some cognitive activities. The possible involvement of genetic factors in human brain sex differentiation is also discussed."

Mixed Studies

- Genetic and epigenetic effects on sexual brain organization mediated by sex hormones
    - "Alterations of sex hormone levels during pre- or perinatal sexual brain organization - responsible for long-term changes of gonadotropin secretion, sexual orientation, and gender role behavior - can be caused by: 1. Genetic effects, i.e. mutations or polymorphisms of a) 21-hydroxylase genes on chromosome 6, b) 3beta-hydroxysteroid dehydrogenase genes in chromosome 1 or c) X-chromosomal genes, and 2. Epigenetic effects, such as a) stressful situations - especially in combination with mutations - and b) endocrine disrupters, e.g. the pesticide DDT and its metabolites, which display estrogenic, antiandrogenic, and inhibitory effects on the enzyme 3beta-hydroxysteroid dehydrogenase leading to increased levels of dehydroepiandrosterone and its sulfate as precursors of endogenous androgens and estrogens. In connection with the introduction and extensive use of the pesticide DDT, the following findings were obtained in subjects born before as compared to those born during this period: 1. The prevalence of patients with polycystic ovaries (PCO), idiopatic oligospermia (IO), and transsexualism (TS) increased significantly (about 3-4 fold). 2. Partial 21-hydroxylase deficiencies were observed in most patients with PCO and TS and some patients with IO born before this period. 3. In contrast, most patients with PCO and TS and several patients with IO born during the period of massive use of DDT displayed clearly increased plasma levels of dehydroepiandrosterone sulfate (DHEA-S) and DHEA-S/cortisol ratios suggesting partial 3beta-hydroxsteroid dehydrogenase (3beta-HSD) deficiencies. Interestingly enough, geneticists could not find any mutations of 3beta-HSD genes in such subjects. However, o,p'-DDT and/or its metabolite o,p'-DDD are strong inhibitors of 3beta-HSD, indicating their possible co-responsibility for such life-long ontogenetic alterations. Finally, some data suggest that endocrine disrupters may also be able to affect the development of sexual orientation."
- A sex difference in the hypothalamic uncinate nucleus: relationship to gender identity

    - “Transsexuality is an individual's unshakable conviction of belonging to the opposite sex, resulting in a request for sex-reassignment surgery. We have shown previously that the bed nucleus of the stria terminalis (BSTc) is female in size and neuron number in male-to-female transsexual people. In the present study we investigated the hypothalamic uncinate nucleus, which is composed of two subnuclei, namely interstitial nucleus of the anterior hypothalamus (INAH) 3 and 4. Post-mortem brain material was used from 42 subjects: 14 control males, 11 control females, 11 male-to-female transsexual people, 1 female-to-male transsexual subject and 5 non-transsexual subjects who were castrated because of prostate cancer. To identify and delineate the nuclei and determine their volume and shape we used three different stainings throughout the nuclei in every 15th section, i.e. thionin, neuropeptide Y and synaptophysin, using an image analysis system. The most pronounced differences were found in the INAH3 subnucleus. Its volume in thionin sections was 1.9 times larger in control males than in females ($P<$ 0.013) and contained 2.3 times as many cells ($P<$0.002). We showed for the first time that INAH3 volume and number of neurons of male-to-female transsexual people is similar to that of control females. The female-to-male transsexual subject had an INAH3 volume and number of neurons within the male control range, even though the treatment with testosterone had been stopped three years before death. The castrated men had an INAH3 volume and neuron number that was intermediate between males (volume and number of neurons $P>$ 0.117) and females (volume $P>$ 0.245 and number of neurons $P>$ 0.341). There was no difference in INAH3 between pre-and post-menopausal women, either in the volume ($P>$ 0.84) or in the number of neurons ($P<$ 0.439), indicating that the feminization of the INAH3 of male-to-female transsexuals was not due to estrogen treatment. We propose that the sex reversal of the INAH3 in transsexual people is at least partly a marker of an early atypical sexual differentiation of the brain and that the changes in INAH3 and the BSTc may belong to a complex network that may structurally and functionally be related to gender identity.”
- Sexual differentiation of the human brain in relation to gender identity and sexual orientation
    - “It is believed that during the intrauterine period the fetal brain develops in the male direction through a direct action of testosterone on the developing nerve cells, or in the female direction through the absence of this hormone surge. According to this concept, our gender identity (the conviction of belonging to the male or female gender) and sexual orientation should be programmed into our brain structures when we are still in the womb. However, since sexual differentiation of the genitals takes place in the first two months of pregnancy and sexual differentiation of the brain starts in the second half of pregnancy, these two processes can be influenced independently, which may result in transsexuality. This also means that in the event of ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain. There is no proof that social environment after birth has an effect on gender identity or sexual orientation. Data on genetic and hormone independent influence on gender identity are presently divergent and do not provide convincing information about the underlying etiology. To what extent fetal programming may determine sexual orientation is also a matter of discussion. A number of studies show patterns of sex atypical cerebral dimorphism in homosexual subjects. Although the crucial question, namely how such complex functions as sexual orientation and identity are processed in the brain

remains unanswered, emerging data point at a key role of specific neuronal circuits involving the hypothalamus."

- Sex differences in the brain, behavior, and neuropsychiatric disorders
    - "Sex differences in the brain are reflected in behavior and in the risk for neuropsychiatric disorders. The fetal brain develops in the male direction due to a direct effect of testosterone on the developing neurons, or in the female direction due to the absence of such a testosterone surge. Because sexual differentiation of the genitals takes place earlier in intrauterine life than sexual differentiation of the brain, these two processes can be influenced independently of each other. Gender identity (the conviction of belonging to the male or female gender), sexual orientation (heterosexuality, homosexuality, or bisexuality), pedophilia, sex differences in cognition, and the risks for neuropsychiatric disorders are programmed into our brains during early development. There is no proof that postnatal social environment has any crucial effect on gender identity or sexual orientation. Structural and functional sex differences in brain areas, together with changes in sex hormone levels and their receptors in development and adulthood, are closely related to sex differences in behavior and neuropsychiatric disorders. Knowing that such a relationship exists may help bring about sex-specific therapeutic strategies."
- Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders
    - "During the intrauterine period a testosterone surge masculinizes the fetal brain, whereas the absence of such a surge results in a feminine brain. As sexual differentiation of the brain takes place at a much later stage in development than sexual differentiation of the genitals, these two processes can be influenced independently of each other. Sex differences in cognition, gender identity (an individual's perception of their own sexual identity), sexual orientation (heterosexuality, homosexuality or bisexuality), and the risks of developing neuropsychiatric disorders are programmed into our brain during early development. There is no evidence that one's postnatal social environment plays a crucial role in gender identity or sexual orientation. We discuss the relationships between structural and functional sex differences of various brain areas and the way they change along with any changes in the supply of sex hormones on the one hand and sex differences in behavior in health and disease on the other."
- Evidence supporting the biologic nature of gender identity
    - "Evidence that there is a biologic basis for gender identity primarily involves (1) data on gender identity in patients with disorders of sex development (DSDs, also known as differences of sex development) along with (2) neuroanatomical differences associated with gender identity."
- Kisspeptin Expression in the Human Infundibular Nucleus in Relation to Sex, Gender Identity, and Sexual Orientation
    - "*Results:* Quantitative analysis confirmed that the human infundibular kisspeptin system exhibits a female-dominant sex difference. The number of kisspeptin neurons is significantly greater in the infant/prepubertal and elderly periods compared with the adult period. Finally, in MTF transsexuals, but not homosexual men, a female-typical kisspeptin expression was observed.
      Conclusions: These findings suggest that infundibular kisspeptin neurons are sensitive to circulating sex steroid hormones throughout life and that the sex reversal observed in MTF transsexuals might reflect, at least partially, an atypical brain sexual differentiation."

- Neural Network of Body Representation Differs between Transsexuals and Cissexuals
    - "Body image is the internal representation of an individual's own physical appearance. Individuals with gender identity disorder (GID), commonly referred to as transsexuals (TXs), are unable to form a satisfactory body image due to the dissonance between their biological sex and gender identity. We reasoned that changes in the resting-state functional connectivity (rsFC) network would neurologically reflect such experiential incongruence in TXs. Using graph theory-based network analysis, we investigated the regional changes of the degree centrality of the rsFC network. The degree centrality is an index of the functional importance of a node in a neural network. We hypothesized that three key regions of the body representation network, i.e., the primary somatosensory cortex, the superior parietal lobule and the insula, would show a higher degree centrality in TXs. Twenty-three pre-treatment TXs (11 male-to-female and 12 female-to-male TXs) as one psychosocial group and 23 age-matched healthy cissexual control subjects (CISs, 11 males and 12 females) were recruited. Resting-state functional magnetic resonance imaging was performed, and binarized rsFC networks were constructed. The TXs demonstrated a significantly higher degree centrality in the bilateral superior parietal lobule and the primary somatosensory cortex. In addition, the connectivity between the right insula and the bilateral primary somatosensory cortices was negatively correlated with the selfness rating of their desired genders. These data indicate that the key components of body representation manifest in TXs as critical function hubs in the rsFC network. The negative association may imply a coping mechanism that dissociates bodily emotion from body image. The changes in the functional connectome may serve as representational markers for the dysphoric bodily self of TXs."

Studies of Transsexuals

- Anatomic variation of the corpus callosum in persons with gender dysphoria
    - "Previous postmortem anatomical studies have demonstrated differences between male and female in the size and shape of the splenium of the corpus callosum. The current study using the magnetic resonance imager compares the corpus callosum in 20 transsexuals and 40 controls to determine if the anatomic variance is related to anatomic sex or gender identity. No statistical differences were found in the cross-sectional areas of the entire corpus callosum, regardless of genetic sex or gender. However, the genetic males did have a larger whole-brain cross-sectional area. Also, even though there was a wide range of differences in shape and size in the splenium, the study found no significant differences between the sexes or between transsexual patients of either sex and the controls."
- A sex difference in the human brain and its relation to transsexuality
    - "TRANSSEXUALS have the strong feeling, often from childhood onwards, of having been born the wrong sex. The possible psycho-genie or biological aetiology of transsexuality has been the subject of debate for many years. Here we show that the volume of the central subdivision of the bed nucleus of the stria terminalis (BSTc), a brain area that is essential for sexual behaviour, is larger in men than in women. A female-sized BSTc was found in male-to-female transsexuals. The size of the BSTc was not influenced by sex hormones in adulthood and was independent of sexual orientation. Our study is the first to show a female brain structure in genetically male transsexuals and supports the

hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones."

- Male-to-Female Transsexuals Have Female Neuron Numbers in a Limbic Nucleus
    - "Transsexuals experience themselves as being of the opposite sex, despite having the biological characteristics of one sex. A crucial question resulting from a previous brain study in male-to-female transsexuals was whether the reported difference according to gender identity in the central part of the bed nucleus of the stria terminalis (BSTc) was based on a neuronal difference in the BSTc itself or just a reflection of a difference in vasoactive intestinal polypeptide inner- vation from the amygdala, which was used as a marker. Therefore, we determined in 42 subjects the number of somatostatin-expressing neurons in the BSTc in relation to sex, sexual orientation, gender identity, and past or present hormonal status. Regardless of sexual orientation, men had almost twice as many somatostatin neurons as women (P ≈ 0.006). The number of neurons in the BSTc of male- to-female transsexuals was similar to that of the females (P ≈ 0.83). In contrast, the neuron number of a female-to-male transsexual was found to be in the male range. Hormone treatment or sex hormone level variations in adulthood did not seem to have influenced BSTc neuron numbers. The present findings of somatostatin neuronal sex differences in the BSTc and its sex reversal in the transsexual brain clearly support the paradigm that in transsexuals sexual differenti- ation of the brain and genitals may go into opposite directions and point to a neurobiological basis of gender identity disorder."
- Phantom Penises In Transsexuals
    - "How the brain constructs one's inner sense of gender identity is poorly understood. On the other hand, the phenomenon of phantom sensations-- the feeling of still having a body-part after amputation--has been much studied. Around 60% of men experience a phantom penis post-penectomy. As transsexuals report a mismatch between their inner gender identity and that of their body, we won-dered what could be learnt from this regarding innate gender-specific body image. We surveyed male-to-female transsexuals regarding the incidence of phantoms post-gender reassignment surgery. Additionally, we asked female-to-male transsexuals if they had ever had the sensation of having a penis when there was not one physically there. In post-operative male-to-female transsexuals the incidence of phantom penises was significantly reduced at 30%. Remarkably, over 60% of female-to-male transsexuals also reported phantom penises. We explain the absence/presence of phantoms here by postulating a mismatch between the brain's hardwired gender-specific body image and the external somatic gender. Further studies along these lines may provide penetrating insights into the question of how nature and nurture interact to produce our brain-based body image."
- Male-to-Female Transsexuals Show Sex-Atypical Hypothalamus Activation When Smelling Odorous Steroids
    - "One working hypothesis behind transsexuality is that the normal sex differentiation of certain hypothalamic networks is altered. We tested this hypothesis by investigating the pattern of cerebral activation in 12 nonhomosexual male-to-female transsexuals (MFTRs) when smelling 4,16-androstadien-3-one (AND) and estra-1,3,5(10),16-tetraen-3-ol (EST). These steroids are reported to activate the hypothalamic networks in a sex-differentiated way. Like in female controls the hypothalamus in MFTRs activated with AND, whereas smelling of EST engaged the amygdala and piriform cortex. Male controls, on the other hand, activated the hypothalamus with EST. However, when restricting the volume of

interest to the hypothalamus activation was detected in MFTR also with EST, and explorative conjunctional analysis revealed that MFTR shared a hypothalamic cluster with women when smelling AND, and with men when smelling EST. Because the EST effect was limited, MFTR differed significantly only from male controls, and only for EST-AIR and EST-AND. These data suggest a pattern of activation away from the biological sex, occupying an intermediate position with predominantly female-like features. Because our MFTRs were nonhomosexual, the results are unlikely to be an effect of sexual practice. Instead, the data implicate that transsexuality may be associated with sex-atypical physiological responses in specific hypothalamic circuits, possibly as a consequence of a variant neuronal differentiation."

- Sex difference in the hypothalamic uncinate nucleus: relationship to gender identity
    - "Transsexuality is an individual's unshakable conviction of belonging to the opposite sex, resulting in a request for sex-reassignment surgery. We have shown previously that the bed nucleus of the stria terminalis (BSTc) is female in size and neuron number in male-to-female transsexual people. In the present study we investigated the hypothalamic uncinate nucleus, which is composed of two subnuclei, namely interstitial nucleus of the anterior hypothalamus (INAH) 3 and 4. Post-mortem brain material was used from 42 subjects: 14 control males, 11 control females, 11 male-to-female transsexual people, 1 female-to-male transsexual subject and 5 non-transsexual subjects who were castrated because of prostate cancer. To identify and delineate the nuclei and determine their volume and shape we used three different stainings throughout the nuclei in every 15th section, i.e. thionin, neuropeptide Y and synaptophysin, using an image analysis system. The most pronounced differences were found in the INAH3 subnucleus. Its volume in thionin sections was 1.9 times larger in control males than in females ($P<$ 0.013) and contained 2.3 times as many cells ($P<0.002$). We showed for the first time that INAH3 volume and number of neurons of male-to-female transsexual people is similar to that of control females. The female-to-male transsexual subject had an INAH3 volume and number of neurons within the male control range, even though the treatment with testosterone had been stopped three years before death. The castrated men had an INAH3 volume and neuron number that was intermediate between males (volume and number of neurons $P>$ 0.117) and females (volume $P>$ 0.245 and number of neurons $P>$ 0.341). There was no difference in INAH3 between pre-and post-menopausal women, either in the volume ($P>$ 0.84) or in the number of neurons ($P<$ 0.439), indicating that the feminization of the INAH3 of male-to-female transsexuals was not due to estrogen treatment. We propose that the sex reversal of the INAH3 in transsexual people is at least partly a marker of an early atypical sexual differentiation of the brain and that the changes in INAH3 and the BSTc may belong to a complex network that may structurally and functionally be related to gender identity."
- Specific cerebral activation due to visual erotic stimuli in male-to-female transsexuals compared with male and female controls: an fMRI study
    - "*Results:* Significantly enhanced activation for men compared with women was revealed in brain areas involved in erotic processing, i.e., the thalamus, the amygdala, and the orbitofrontal and insular cortex, whereas no specific activation for women was found. When comparing MTF transsexuals with male volunteers, activation patterns similar to female volunteers being compared with male volunteers were revealed. Sexual arousal was assessed using standard rating scales and did not differ significantly for the three

groups.
Conclusions: We revealed a cerebral activation pattern in MTF transsexuals compared with male controls similar to female controls compared with male controls during viewing of erotic stimuli, indicating a tendency of female-like cerebral processing in transsexualism."

- Regional gray matter variation in male-to-female transsexualism
    - "Gender identity—one's sense of being a man or a woman—is a fundamental perception experienced by all individuals that extends beyond biological sex. Yet, what contributes to our sense of gender remains uncertain. Since individuals who identify as transsexual report strong feelings of being the opposite sex and a belief that their sexual characteristics do not reflect their true gender, they constitute an invaluable model to understand the biological underpinnings of gender identity. We analyzed MRI data of 24 male-to-female (MTF) transsexuals not yet treated with cross-sex hormones in order to determine whether gray matter volumes in MTF transsexuals more closely resemble people who share their biological sex (30 control men), or people who share their gender identity (30 control women). Results revealed that regional gray matter variation in MTF transsexuals is more similar to the pattern found in men than in women. However, MTF transsexuals show a significantly larger volume of regional gray matter in the right putamen compared to men. These findings provide new evidence that transsexualism is associated with distinct cerebral pattern, which supports the assumption that brain anatomy plays a role in gender identity."
- The microstructure of white matter in male to female transsexuals before cross-sex hormonal treatment. A DTI study
    - "*Results:* MtF transsexuals differed from both male and female controls bilaterally in the superior longitudinal fasciculus, the right anterior cingulum, the right forceps minor, and the right corticospinal tract.
      Conclusions: Our results show that the white matter microstructure pattern in untreated MtF transsexuals falls halfway between the pattern of male and female controls. The nature of these differences suggests that some fasciculi do not complete the masculinization process in MtF transsexuals during brain development."
- White matter microstructure in female to male transsexuals before cross-sex hormonal treatment. A diffusion tensor imaging study
    - "Results: In controls, males have significantly higher FA values than females in the medial and posterior parts of the right superior longitudinal fasciculus (SLF), the forceps minor, and the corticospinal tract. Compared to control females, FtM showed higher FA values in posterior part of the right SLF, the forceps minor and corticospinal tract. Compared to control males, FtM showed only lower FA values in the corticospinal tract.
      Conclusions: Our results show that the white matter microstructure pattern in untreated FtM transsexuals is closer to the pattern of subjects who share their gender identity (males) than those who share their biological sex (females). Our results provide evidence for an inherent difference in the brain structure of FtM transsexuals."
- Sex Dimorphism of the Brain in Male-to-Female Transsexuals
    - "Gender dysphoria is suggested to be a consequence of sex atypical cerebral differentiation. We tested this hypothesis in a magnetic resonance study of voxel-based morphometry and structural volumetry in 48 heterosexual men (HeM) and women (HeW) and 24 gynephillic male to female transsexuals (MtF-TR). Specific interest was paid to

gray matter (GM) and white matter (WM) fraction, hemispheric asymmetry, and volumes of the hippocampus, thalamus, caudate, and putamen. Like HeM, MtF-TR displayed larger GM volumes than HeW in the cerebellum and lingual gyrus and smaller GM and WM volumes in the precentral gyrus. Both male groups had smaller hippocampal volumes than HeW. As in HeM, but not HeW, the right cerebral hemisphere and thalamus volume was in MtF-TR lager than the left. None of these measures differed between HeM and MtF-TR. MtF-TR displayed also singular features and differed from both control groups by having reduced thalamus and putamen volumes and elevated GM volumes in the right insular and inferior frontal cortex and an area covering the right angular gyrus.The present data do not support the notion that brains of MtF-TR are feminized. The observed changes in MtF-TR bring attention to the networks inferred in processing of body perception."

- Intrinsic cerebral connectivity analysis in an untreated female-to-male transsexual subject: a first attempt using resting-state fMRI
    - "Results: Brain areas sensitive to gender dimorphism like left lingual gyrus and precuneus showed strong similarities between the FtM subject and female control group with respect to control males, with comparable extension and location of functional connectivity maps. ROI analysis confirmed this evidence, highlighting a greater pattern of differences between the FtM subject and males and the FtM subject and females. No difference between seed-voxel results in the FtM subject and females was found.
      Conclusions: These data partially support the idea that untreated FtM transgender shows a functional connectivity profile comparable to female control subjects."
- Regional Grey Matter Structure Differences between Transsexuals and Healthy Controls—A Voxel Based Morphometry Study
    - "Gender identity disorder (GID) refers to transsexual individuals who feel that their assigned biological gender is incongruent with their gender identity and this cannot be explained by any physical intersex condition. There is growing scientific interest in the last decades in studying the neuroanatomy and brain functions of transsexual individuals to better understand both the neuroanatomical features of transsexualism and the background of gender identity. So far, results are inconclusive but in general, transsexualism has been associated with a distinct neuroanatomical pattern. Studies mainly focused on male to female (MTF) transsexuals and there is scarcity of data acquired on female to male (FTM) transsexuals. Thus, our aim was to analyze structural MRI data with voxel based morphometry (VBM) obtained from both FTM and MTF transsexuals (n=17) and compare them to the data of 18 age matched healthy control subjects (both males and females). We found differences in the regional grey matter (GM) structure of transsexual compared with control subjects, independent from their biological gender, in the cerebellum, the left angular gyrus and in the left inferior parietal lobule. Additionally, our findings showed that in several brain areas, regarding their GM volume, transsexual subjects did not differ significantly from controls sharing their gender identity but were different from those sharing their biological gender (areas in the left and right precentral gyri, the left postcentral gyrus, the left posterior cingulate, precuneus and calcarinus, the right cuneus, the right fusiform, lingual, middle and inferior occipital, and inferior temporal gyri). These results support the notion that structural brain differences exist between transsexual and healthy control subjects and that majority of these structural differences are dependent on the biological gender."

- Cortical Thickness in Untreated Transsexuals | Cerebral Cortex
    - "Sex differences in cortical thickness (CTh) have been extensively investigated but as yet there are no reports on CTh in transsexuals. Our aim was to determine whether the CTh pattern in transsexuals before hormonal treatment follows their biological sex or their gender identity. We performed brain magnetic resonance imaging on 94 subjects: 24 untreated female-to-male transsexuals (FtMs), 18 untreated male-to-female transsexuals (MtFs), and 29 male and 23 female controls in a 3-T TIM-TRIO Siemens scanner. T_1-weighted images were analyzed to obtain CTh and volumetric subcortical measurements with FreeSurfer software. CTh maps showed control females have thicker cortex than control males in the frontal and parietal regions. In contrast, males have greater right putamen volume. FtMs had a similar CTh to control females and greater CTh than males in the parietal and temporal cortices. FtMs had larger right putamen than females but did not differ from males. MtFs did not differ in CTh from female controls but had greater CTh than control males in the orbitofrontal, insular, and medial occipital regions. In conclusion, FtMs showed evidence of subcortical gray matter masculinization, while MtFs showed evidence of CTh feminization. In both types of transsexuals, the differences with respect to their biological sex are located in the right hemisphere."
- Brain Signature Characterizing the Body-Brain-Mind Axis of Transsexuals
    - "Individuals with gender identity disorder (GID), who are commonly referred to as transsexuals (TXs), are afflicted by negative psychosocial stressors. Central to the psychological complex of TXs is the conviction of belonging to the opposite sex. Neuroanatomical and functional brain imaging studies have demonstrated that the GID is associated with brain alterations. In this study, we found that TXs identify, when viewing male-female couples in erotic or non-erotic ("neutral") interactions, with the couple member of the desired gender in both situations. By means of functional magnetic resonance imaging, we found that the TXs, as opposed to controls (CONs), displayed an increased functional connectivity between the ventral tegmental area, which is associated with dimorphic genital representation, and anterior cingulate cortex subregions, which play a key role in social exclusion, conflict monitoring and punishment adjustment. The neural connectivity pattern suggests a brain signature of the psychosocial distress for the gender-sex incongruity of TXs."
- White matter microstructure in transsexuals and controls investigated by diffusion tensor imaging
    - "Biological causes underpinning the well known gender dimorphisms in human behavior, cognition, and emotion have received increased attention in recent years. The advent of diffusion-weighted magnetic resonance imaging has permitted the investigation of the white matter microstructure in unprecedented detail. Here, we aimed to study the potential influences of biological sex, gender identity, sex hormones, and sexual orientation on white matter microstructure by investigating transsexuals and healthy controls using diffusion tensor imaging (DTI). Twenty-three female-to-male (FtM) and 21 male-to-female (MtF) transsexuals, as well as 23 female (FC) and 22 male (MC) controls underwent DTI at 3 tesla. Fractional anisotropy, axial, radial, and mean diffusivity were calculated using tract-based spatial statistics (TBSS) and fiber tractography. Results showed widespread significant differences in mean diffusivity between groups in almost all white matter tracts. FCs had highest mean diffusivities, followed by FtM transsexuals with lower values, MtF transsexuals with further reduced values, and MCs with lowest

values. Investigating axial and radial diffusivities showed that a transition in axial diffusivity accounted for mean diffusivity results. No significant differences in fractional anisotropy maps were found between groups. Plasma testosterone levels were strongly correlated with mean, axial, and radial diffusivities. However, controlling for individual estradiol, testosterone, or progesterone plasma levels or for subjects' sexual orientation did not change group differences. Our data harmonize with the hypothesis that fiber tract development is influenced by the hormonal environment during late prenatal and early postnatal brain development."

- Structural Connectivity Networks of Transgender People | Cerebral Cortex
    - "Although previous investigations of transsexual people have focused on regional brain alterations, evaluations on a network level, especially those structural in nature, are largely missing. Therefore, we investigated the structural connectome of 23 female-to-male (FtM) and 21 male-to-female (MtF) transgender patients before hormone therapy as compared with 25 female and 25 male healthy controls. Graph theoretical analysis of whole-brain probabilistic tractography networks (adjusted for differences in intracranial volume) showed decreased hemispheric connectivity ratios of subcortical/limbic areas for both transgender groups. Subsequent analysis revealed that this finding was driven by increased interhemispheric lobar connectivity weights (LCWs) in MtF transsexuals and decreased intrahemispheric LCWs in FtM patients. This was further reflected on a regional level, where the MtF group showed mostly increased local efficiencies and FtM patients decreased values. Importantly, these parameters separated each patient group from the remaining subjects for the majority of significant findings. This work complements previously established regional alterations with important findings of structural connectivity. Specifically, our data suggest that network parameters may reflect unique characteristics of transgender patients, whereas local physiological aspects have been shown to represent the transition from the biological sex to the actual gender identity."
- Hypothalamic Response to the Chemo-Signal Androstadienone in Gender Dysphoric Children and Adolescents
    - "The odorous steroid androstadienone, a putative male chemo-signal, was previously reported to evoke sex differences in hypothalamic activation in adult heterosexual men and women. In order to investigate whether puberty modulated this sex difference in response to androstadienone, we measured the hypothalamic responsiveness to this chemo-signal in 39 pre-pubertal and 41 adolescent boys and girls by means of functional magnetic resonance imaging. We then investigated whether 36 pre-pubertal children and 38 adolescents diagnosed with gender dysphoria (GD; DSM-5) exhibited sex-atypical (in accordance with their experienced gender), rather than sex-typical (in accordance with their natal sex) hypothalamic activations during olfactory stimulation with androstadienone. We found that the sex difference in responsiveness to androstadienone was already present in pre-pubertal control children and thus likely developed during early perinatal development instead of during sexual maturation. Adolescent girls and boys with GD both responded remarkably like their experienced gender, thus sex-atypical. In contrast, pre-pubertal girls with GD showed neither a typically male nor female hypothalamic activation pattern and pre-pubertal boys with GD had hypothalamic activations in response to androstadienone that were similar to control boys, thus sex-typical. We present here a unique data set of boys and girls diagnosed with GD at two

different developmental stages, showing that these children possess certain sex-atypical functional brain characteristics and may have undergone atypical sexual differentiation of the brain."

- Cerebral serotonin transporter asymmetry in females, males and male-to-female transsexuals measured by PET in vivo
    - "The serotonergic system modulates brain functions that are considered to underlie affective states, emotion and cognition. Several lines of evidence point towards a strong lateralization of these mental processes, which indicates similar asymmetries in associated neurotransmitter systems. Here, our aim was to investigate a potential asymmetry of the serotonin transporter distribution using positron emission tomography and the radioligand [(11)C]DASB in vivo. As brain asymmetries may differ between sexes, we further aimed to compare serotonin transporter asymmetry between females, males and male-to-female (MtF) transsexuals whose brains are considered to be partly feminized. Voxel-wise analysis of serotonin transporter binding in all groups showed both strong left and rightward asymmetries in several cortical and subcortical structures including temporal and frontal cortices, anterior cingulate, hippocampus, caudate and thalamus. Further, male controls showed a rightward asymmetry in the midcingulate cortex, which was absent in females and MtF transsexuals. The present data support the notion of a lateralized serotonergic system, which is in line with previous findings of asymmetric serotonin-1A receptor distributions, extracellular serotonin concentrations, serotonin turnover and uptake. The absence of serotonin transporter asymmetry in the midcingulate in MtF transsexuals may be attributed to an absence of brain masculinization in this region."
- Structural Connectivity Networks of Transgender People
    - "Although previous investigations of transsexual people have focused on regional brain alterations, evaluations on a network level, especially those structural in nature, are largely missing. Therefore, we investigated the structural connectome of 23 female-to-male (FtM) and 21 male-to-female (MtF) transgender patients before hormone therapy as compared with 25 female and 25 male healthy controls. Graph theoretical analysis of whole-brain probabilistic tractography networks (adjusted for differences in intracranial volume) showed decreased hemispheric connectivity ratios of subcortical/limbic areas for both transgender groups. Subsequent analysis revealed that this finding was driven by increased interhemispheric lobar connectivity weights (LCWs) in MtF transsexuals and decreased intrahemispheric LCWs in FtM patients. This was further reflected on a regional level, where the MtF group showed mostly increased local efficiencies and FtM patients decreased values. Importantly, these parameters separated each patient group from the remaining subjects for the majority of significant findings. This work complements previously established regional alterations with important findings of structural connectivity. Specifically, our data suggest that network parameters may reflect unique characteristics of transgender patients, whereas local physiological aspects have been shown to represent the transition from the biological sex to the actual gender identity."
- Regional volumes and spatial volumetric distribution of gray matter in the gender dysphoric brain
    - "The sexual differentiation of the brain is primarily driven by gonadal hormones during fetal development. Leading theories on the etiology of gender dysphoria (GD) involve

deviations herein. To examine whether there are signs of a sex-atypical brain development in GD, we quantified regional neural gray matter (GM) volumes in 55 female-to-male and 38 male-to-female adolescents, 44 boys and 52 girls without GD and applied both univariate and multivariate analyses. In girls, more GM volume was observed in the left superior medial frontal cortex, while boys had more volume in the bilateral superior posterior hemispheres of the cerebellum and the hypothalamus. Regarding the GD groups, at whole-brain level they differed only from individuals sharing their gender identity but not from their natal sex. Accordingly, using multivariate pattern recognition analyses, the GD groups could more accurately be automatically discriminated from individuals sharing their gender identity than those sharing their natal sex based on spatially distributed GM patterns. However, region of interest analyses indicated less GM volume in the right cerebellum and more volume in the medial frontal cortex in female-to-males in comparison to girls without GD, while male-to-females had less volume in the bilateral cerebellum and hypothalamus than natal boys. Deviations from the natal sex within sexually dimorphic structures were also observed in the untreated subsamples. Our findings thus indicate that GM distribution and regional volumes in GD adolescents are largely in accordance with their respective natal sex. However, there are subtle deviations from the natal sex in sexually dimorphic structures, which can represent signs of a partial sex-atypical differentiation of the brain."

- Neuroimaging studies in people with gender incongruence
    - "The current review gives an overview of brain studies in transgender people. First, we describe studies into the aetiology of feelings of gender incongruence, primarily addressing the sexual differentiation hypothesis: does the brain of transgender individuals resemble that of their natal sex, or that of their experienced gender? Findings from neuroimaging studies focusing on brain structure suggest that the brain phenotypes of trans women (MtF) and trans men (FtM) differ in various ways from control men and women with feminine, masculine, demasculinized and defeminized features. The brain phenotypes of people with feelings of gender incongruence may help us to figure out whether sex differentiation of the brain is atypical in these individuals, and shed light on gender identity development. Task-related imaging studies may show whether brain activation and task performance in transgender people is sex-atypical. Second, we review studies that evaluate the effects of cross-sex hormone treatment on the brain. This type of research provides knowledge on how changes in sex hormone levels may affect brain structure and function."
- Male–to–female transsexuals have female neuron numbers in a limbic nucleus
    - "Transsexuals experience themselves as being of the opposite sex, despite having the biological characteristics of one sex. A crucial question resulting from a previous brain study in male-to-female transsexuals was whether the reported difference according to gender identity in the central part of the bed nucleus of the stria terminalis (BSTc) was based on a neuronal difference in the BSTc itself or just a reflection of a difference in vasoactive intestinal polypeptide innervation from the amygdala, which was used as a marker. Therefore, we determined in 42 subjects the number of somatostatin-expressing neurons in the BSTc in relation to sex, sexual orientation, gender identity, and past or present hormonal status. Regardless of sexual orientation, men had almost twice as many somatostatin neurons as women ($P < 0.006$). The number of neurons in the BSTc of male-to-female transsexuals was similar to that of the females ($P = 0.83$). In contrast,

the neuron number of a female-to-male transsexual was found to be in the male range. Hormone treatment or sex hormone level variations in adulthood did not seem to have influenced BSTc neuron numbers. The present findings of somatostatin neuronal sex differences in the BSTc and its sex reversal in the transsexual brain clearly support the paradigm that in transsexuals sexual differentiation of the brain and genitals may go into opposite directions and point to a neurobiological basis of gender identity disorder."

- Regional cerebral blood flow changes in female to male gender identity disorder
    - *"Results:* GID subjects had a significant decrease in rCBF in the left anterior cingulate cortex (ACC) and a significant increase in the right insula compared to control subjects.
      Conclusions: The ACC and insula are regions that have been noted as being related to human sexual behavior and consciousness. From these findings, useful insights into the biological basis of GID were suggested."
- Male Gender Identity in Complete Androgen Insensitivity Syndrome
    - "Women and girls with complete androgen insensitivity syndrome (CAIS) invariably have a female typical core gender identity. In this case report, we describe the first case of male gender identity in a CAIS individual raised female leading to complete sex reassignment involving both androgen treatment and phalloplasty. CAIS was diagnosed at age 17, based on an unambiguously female phenotype, a 46,XY karyotype, and a 2660delT androgen receptor (AR) gene mutation, leading to a premature stop in codon 807. Bilateral gonadectomy was performed but a short period of estrogen treatment induced a negative emotional reaction and treatment was stopped. Since the age of 3, childhood-onset cross gender behavior had been noticed. After a period of psychotherapy, persisting male gender identity was confirmed. There was no psychiatric co-morbidity and there was an excellent real life experience. Testosterone substitution was started, however without inducing any of the desired secondary male characteristics. A subcutaneous mastectomy was performed and the patient received phalloplasty by left forearm free flap and scrotoplasty. Testosterone treatment was continued, without inducing virilization, and bone density remained normal. The patient qualifies as female-to-male transsexual and was treated according to the Standards of Care by the World Professional Association for Transgender Health with good outcome. However, we do not believe that female sex of rearing as a standard procedure should be questioned in CAIS. Our case challenges the role of a functional AR pathway in the development of male gender identity."
- Changing your sex changes your brain: influences of testosterone and estrogen on adult human brain structure
    - "*Results*: Compared with controls, anti-androgen + estrogen treatment decreased brain volumes of male-to-female subjects towards female proportions, while androgen treatment in female-to-male subjects increased total brain and hypothalamus volumes towards male proportions.
      Conclusions: The findings suggest that, throughout life, gonadal hormones remain essential for maintaining aspects of sex-specific differences in the human brain."
- Neuroimaging Differences in Spatial Cognition between Men and Male-to-Female Transsexuals Before and During Hormone Therapy
    - "*Results:* The classical mental rotation network was activated in all three groups, but significant differences within this network were observed. Men without GID exhibited significantly greater activation of the left parietal cortex (BA 40), a key region for mental

rotation processes. Both transsexual groups revealed stronger activation of temporo-occipital regions in comparison with men without GID.
Conclusions: Our results confirmed previously reported deviances of brain activation patterns in transsexual men from men without GID and also corroborated these findings in a group of transsexual patients receiving cross-sex hormone therapy. The present study indicates that there are a priori differences between men and transsexual patients caused by different neurobiological processes or task-solving strategies and that these differences remain stable over the course of hormonal treatment."

- Increased Cortical Thickness in Male-to-Female Transsexualism
    - "*Results:* Results revealed thicker cortices in MTF transsexuals, both within regions of the left hemisphere (i.e., frontal and orbito-frontal cortex, central sulcus, perisylvian regions, pa- racentral gyrus) and right hemisphere (i.e., pre-/post-central gyrus, parietal cortex, temporal cortex, precu- neus, fusiform, lingual, and orbito-frontal gyrus).
    Conclusion: These findings provide further evidence that brain anatomy is associated with gender identity, where measures in MTF transsexuals appear to be shifted away from gender-congruent men."

Hormonal Influences on Gender Identity and Sexed Behaviors

Human Studies

- An endocrine disrupting chemical, bisphenol A: could it be associated with sex differentiation in brain regarding to transsexuality? | ECE2015 | 17th European
    - "Transsexuality is characterised by a belief of having been born in a wrong body. Sexual differentiation of genitals take place in the first 2 months of pregnancy. Sexual differentiation of the brain takes place in the second half of pregnancy. It is found that there is structural sex differences in the central nucleus of the bed nucleus of the stria terminalis (BSTc). Structural differences were found to be reversed in transsexual people. In humans, the main mechanism appears to involve a direct effect of testosterone on the developing brain. Direct effect of testosterone on the developing brain in boys and lack of this effect in girls are crucial factors in the development of male and female gender identity. The origin of transsexuality is based on the fact that the differentiation of sexual organs takes place before the sexual differentiation of the brain. A Reversal was found in BSTc. In men this area is twice the size of that in women. In male-to-female transsexuals they found female BSTc. They had shown that sex reversal of the differences in the BSTc were independent of changing hormone levels in adulthood. The size of BSTc and the number of neurons match the gender that transsexuals feel they belong to, not the sex of their sexual organs. An endocrine disrupting chemical (EDC), bisphenol A (BPA), acts as oestrogen mimic compound. BPA may affect sexual differentiation of brain and cause reversal of differentiation in male to female transsexual as female brain. Brain expresses the oestrogen receptors and other hormone receptors making it a potential target for EDC. Transsexuality presume a combination of a genetic background and an early effect on interaction of sex hormones with developing brain during critical foetal period. We hypothesize that exposure to BPA may be a cause for transsexualism."
- Early androgen exposure and human gender development

- "During early development, testosterone plays an important role in sexual differentiation of the mammalian brain and has enduring influences on behavior. Testosterone exerts these influences at times when the testes are active, as evidenced by higher concentrations of testosterone in developing male than in developing female animals. This article critically reviews the available evidence regarding influences of testosterone on human gender-related development. In humans, testosterone is elevated in males from about weeks 8 to 24 of gestation and then again during early postnatal development. Individuals exposed to atypical concentrations of testosterone or other androgenic hormones prenatally, for example, because of genetic conditions or because their mothers were prescribed hormones during pregnancy, have been consistently found to show increased male-typical juvenile play behavior, alterations in sexual orientation and gender identity (the sense of self as male or female), and increased tendencies to engage in physically aggressive behavior. Studies of other behavioral outcomes following dramatic androgen abnormality prenatally are either too small in their numbers or too inconsistent in their results, to provide similarly conclusive evidence. Studies relating normal variability in testosterone prenatally to subsequent gender-related behavior have produced largely inconsistent results or have yet to be independently replicated. For studies of prenatal exposures in typically developing individuals, testosterone has been measured in single samples of maternal blood or amniotic fluid. These techniques may not be sufficiently powerful to consistently detect influences of testosterone on behavior, particularly in the relatively small samples that have generally been studied. The postnatal surge in testosterone in male infants, sometimes called mini-puberty, may provide a more accessible opportunity for measuring early androgen exposure during typical development. This approach has recently begun to be used, with some promising results relating testosterone during the first few months of postnatal life to later gender-typical play behavior. In replicating and extending these findings, it may be important to assess testosterone when it is maximal (months 1 to 2 postnatal) and to take advantage of the increased reliability afforded by repeated sampling."

- PREDICTING GENDER DYSPHORIA IN CHILDREN AND WOMEN WITH CONGENITAL ADRENAL HYPERPLASIA
    - "Understanding how gender identity develops has important theoretical implications for typically developing populations and clinical implications for those with gender variability or dysphoria (e.g. low contentment with one's assigned gender) and practical implications for the assignment and management of gender in children born with ambiguous sex/genitalia. While chromosomal females exposed to heightened levels of androgens prenatally consistently express later masculinised/defeminised gendered behaviour, the relationship between prenatal androgen exposure and gender identity development (masculinisation/defeminisation and feelings associated with this) is less consistent. Between-group differences in gender identity, gendered behaviour, gender typicality, gender contentedness, and felt pressure for gender conformity were examined in a sample of children (age 7 – 11) with congenital adrenal hyperplasia (CAH; 23 males, 26 females) together with unaffected siblings (19 males, 29 females). Analyses reveal reduced gender typicality and gender contentedness in girls with CAH together with reduced female gender identity and increased cross-sex gendered behaviour. Bootstrapping mediation analysis examining gender typicality, gender contentedness, and gendered behaviour as mediators between prenatal androgen exposure typicality and

gender identity revealed two important relationships: (1) gendered behaviour mediated the relationship between prenatal androgen exposure typicality and gender identity and, (2) this mediation was moderated by gender typicality and gender contentedness. These findings suggest that, for girls with CAH, the increased expression of cross-sex gendered behaviour contributes to the development of desires to be the other sex and that this relationship may depend upon feelings of gender typicality and contentedness."

- Gendered Development - Hines - - Major Reference Works
    - "How does human behavior come to be gendered, and how do gendered behaviors change or remain stable over time? Although men and women, as well as girls and boys, are largely similar psychologically and behaviorally, there are some areas of gender difference. These include gender identity; sexual orientation; childhood play behaviors, such as toy, playmate, and activity preferences; personality characteristics, such as aggression and empathy; and some specific spatial, mathematical, and verbal abilities. The incidence of many psychiatric disorders also differs by sex. These gender differences appear to result from numerous factors and their interactions. These include genetic information on the sex chromosomes; concentrations of gonadal steroids, particularly testosterone, before and shortly after birth; socialization by parents, peers, teachers, and strangers; and cognitive developmental processes. Gender identity also is a mechanism for acquiring gendered behavior; based on this identity, children self-socialize gendered behavior. These factors have been shown to act individually to influence gendered outcomes. They are also likely to interact with one another to shape gender development, but little research has investigated these interactions. An understanding of gendered development is important for addressing differences between the sexes in social roles and economic status, and should also be relevant to understanding and ameliorating psychiatric disorders that differ by gender. A complete understanding will probably require developmental systems approaches to understanding change and stability over time, but, thus far, such approaches have been uncommon."
- Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: Evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys
    - "The masculinizing effects of prenatal androgens on human neurobehavioral development are well established. Also, the early postnatal surge of androgens in male infants, or *mini-puberty*, has been well documented and is known to influence physiological development, including penile growth. However, neurobehavioral effects of androgen exposure during mini-puberty are largely unknown. The main aim of the current study was to evaluate possible neurobehavioral consequences of mini-puberty by relating penile growth in the early postnatal period to subsequent behavior. Using multiple linear regression, we demonstrated that penile growth between birth and three months postnatal, concurrent with mini-puberty, significantly predicted increased masculine/decreased feminine behavior assessed using the Pre-school Activities Inventory (PSAI) in 81 healthy boys at 3 to 4 years of age. When we controlled for other potential influences on masculine/feminine behavior and/or penile growth, including variance in androgen exposure prenatally and body growth postnatally, the predictive value of penile growth in the early postnatal period persisted. More specifically, prenatal androgen exposure, reflected in the measurement of anogenital distance (AGD), and early postnatal androgen exposure, reflected in penile growth from birth to 3 months, were

significant predictors of increased masculine/decreased feminine behavior, with each accounting for unique variance. Our findings suggest that independent associations of PSAI with AGD at birth and with penile growth during mini-puberty reflect prenatal and early postnatal androgen exposures respectively. Thus, we provide a novel and readily available approach for assessing effects of early androgen exposures, as well as novel evidence that early postnatal aes human neurobehavioral development."

- Atención psicomédica en la disforia de identidad de género durante la adolescencia (Psychomedical care in gender identity dysphoria during adolescence)
    - "There is evidence of a hormonal impact on the etiology of gender identity dysphoria and an underestimation of its prevalence.
      Relevance to DSM-V, including the replacement of the term «gender identity disorder» by «dysphoria gender identity», and thus the partial removal of the previous disease connotation.
      The seventh edition of the international standards World Professional Association for Transgender Health highlight the role of the therapist for advice on the way to the transition.
      The Spanish 2012 guide stands out for its wealth of details and explanations, with a language targeted at different professionals.
      Dysphoria gender identity must be studied by a multidisciplinary team, in which the psychotherapist must be expert in developmental psychopathology and evaluate emotional and behavioral problems."
- Brain responses to sexual images in 46,XY women with complete androgen insensitivity syndrome are female-typical
    - "Androgens, estrogens, and sex chromosomes are the major influences guiding sex differences in brain development, yet their relative roles and importance remain unclear. Individuals with complete androgen insensitivity syndrome (CAIS) offer a unique opportunity to address these issues. Although women with CAIS have a Y chromosome, testes, and produce male-typical levels of androgens, they lack functional androgen receptors preventing responding to their androgens. Thus, they develop a female physical phenotype, are reared as girls, and develop into women. Because sexually differentiated brain development in primates is determined primarily by androgens, but may be affected by sex chromosome complement, it is currently unknown whether brain structure and function in women with CAIS is more like that of women or men. In the first functional neuroimaging study of (46,XY) women with CAIS, typical (46,XX) women, and typical (46, XY) men, we found that men showed greater amygdala activation to sexual images than did either typical women or women with CAIS. Typical women and women with CAIS had highly similar patterns of brain activation, indicating that a Y chromosome is insufficient for male-typical human brain responses. Because women with CAIS produce male-typical or elevated levels of testosteronewhich is aromatized to estradiol these results rule out aromatization of testosterone to estradiol as a determinate of sex differences in patterns of brain activation to sexual images. We cannot, however, rule out an effect of social experience on the brain responses of women with CAIS as all were raised as girls."
- Exposure to prenatal life events stress is associated with masculinized play behavior in girls
    - "Previous research has shown that prenatal exposure to endocrine-disrupting chemicals can alter children's neurodevelopment, including sex-typed behavior, and that it can do so

in different ways in males and females. Non-chemical exposures, including psychosocial stress, may disrupt the prenatal hormonal milieu as well. To date, only one published study has prospectively examined the relationship between exposure to prenatal stress and gender-specific play behavior during childhood, finding masculinized play behavior in girls who experienced high prenatal life events stress, but no associations in boys. Here we examine this question in a second prospective cohort from the Study for Future Families. Pregnant women completed questionnaires on stressful life events during pregnancy, and those who reported one or more events were considered "stressed". Families were recontacted several years later (mean age of index child: 4.9 years), and mothers completed a questionnaire including the validated Preschool Activities Inventory (PSAI), which measures sexually dimorphic play behavior. In sex-stratified analyses, after adjusting for child's age, parental attitudes toward gender-atypical play, age and sex of siblings, and other relevant covariates, girls (n=72) exposed to prenatal life events stress had higher scores on the PSAI masculine sub-scale (β=3.48, p=0.006) and showed a trend toward higher (more masculine) composite scores (β=2.63, p=0.08). By contrast, in males (n=74), there was a trend toward an association between prenatal stress and higher PSAI feminine sub-scale scores (β=2.23, p=0.10), but no association with masculine or composite scores. These data confirm previous findings in humans and animal models suggesting that prenatal stress is a non-chemical endocrine disruptor that may have androgenic effects on female fetuses and anti-androgenic effects on male fetuses."

- Increased Cross-Gender Identification Independent of Gender Role Behavior in Girls with Congenital Adrenal Hyperplasia: Results from a Standardized Assessment of 4- to 11-Year-Old Children
    - "While reports showing a link between prenatal androgen exposure and human gender role behavior are consistent and the effects are robust, associations to gender identity or cross-gender identification are less clear. The aim of the current study was to investigate potential cross-gender identification in girls exposed prenatally to high concentrations of androgens due to classical congenital adrenal hyperplasia (CAH). Assessment included two standardized measures and a short parent interview assessing frequency of behavioral features of cross-gender identification as conceptualized in Part A of the diagnostic criteria for gender identity disorder (GID) in the DSM-IV-TR. Next, because existing measures may have conflated gender role behavior with gender identity and because the distinction is potentially informative, we factor analyzed items from the measures which included both gender identity and gender role items to establish the independence of the two constructs. Participants were 43 girls and 38 boys with CAH and 41 unaffected female and 31 unaffected male relatives, aged 4- to 11-years. Girls with CAH had more cross-gender responses than female controls on all three measures of cross-gender identification as well as on a composite measure of gender identity independent of gender role behavior. Furthermore, parent report indicated that 5/39 (12.8%) of the girls with CAH exhibited cross-gender behavior in all five behavioral domains which comprise the cross-gender identification component of GID compared to 0/105 (0.0%) of the children in the other three groups combined. These data suggest that girls exposed to high concentrations of androgens prenatally are more likely to show cross-gender identification than girls without CAH or boys with and without CAH. Our findings suggest that prenatal androgen exposure could play a role in gender identity

development in healthy children, and may be relevant to gender assignment in cases of prenatal hormone disruption, including, in particular, cases of severely virilized 46,XX CAH."

- Neural Activation During Mental Rotation in Complete Androgen Insensitivity Syndrome: the Influence of Sex Hormones and Sex Chromosomes
    - "Sex hormones, androgens in particular, are hypothesized to play a key role in the sexual differentiation of the human brain. However, possible direct effects of the sex chromosomes, that is, XX or XY, have not been well studied in humans. Individuals with complete androgen insensitivity syndrome (CAIS), who have a 46,XY karyotype but a female phenotype due to a complete androgen resistance, enable us to study the separate effects of gonadal hormones versus sex chromosomes on neural sex differences. Therefore, in the present study, we compared 46,XY men (n = 30) and 46,XX women (n = 29) to 46,XY individuals with CAIS (n = 21) on a mental rotation task using functional magnetic resonance imaging. Previously reported sex differences in neural activation during mental rotation were replicated in the control groups, with control men showing more activation in the inferior parietal lobe than control women. Individuals with CAIS showed a female-like neural activation pattern in the parietal lobe, indicating feminization of the brain in CAIS. Furthermore, this first neuroimaging study in individuals with CAIS provides evidence that sex differences in regional brain function during mental rotation are most likely not directly driven by genetic sex, but rather reflect gonadal hormone exposure."
- Otoacoustic emissions, auditory evoked potentials and self-reported gender in people affected by disorders of sex development (DSD)
    - "Both otoacoustic emissions (OAEs) and auditory evoked potentials (AEPs) are sexually dimorphic, and both are believed to be influenced by prenatal androgen exposure. OAEs and AEPs were collected from people affected by 1 of 3 categories of disorders of sex development (DSD) — (1) women with complete androgen insensitivity syndrome (CAIS); (2) women with congenital adrenal hyperplasia(CAH); and (3) individuals with 46,XY DSD including prenatal androgen exposure who developed a male gender despite initial rearing as females (men with DSD). Gender identity (GI) and role (GR) were measured both retrospectively and at the time of study participation, using standardized questionnaires. The main objective of this study was to determine if patterns of OAEs and AEPs correlate with gender in people affected by DSD and in controls. A second objective was to assess if OAE and AEP patterns differed according to degrees of prenatal androgen exposure across groups. Control males, men with DSD, and women with CAH produced fewer spontaneous OAEs (SOAEs) – the male-typical pattern – than control females and women with CAIS. Additionally, the number of SOAEs produced correlated with gender development across all groups tested. Although some sex differences in AEPs were observed between control males and females, AEP measures did not correlate with gender development, nor did they vary according to degrees of prenatal androgen exposure, among people with DSD. Thus, OAEs, but not AEPs, may prove useful as bioassays for assessing early brain exposure to androgens and predicting gender development in people with DSD."
- Postnatal Testosterone Concentrations and Male Social Development
    - "Converging evidence from over 40 years of behavioral research indicates that higher testicular androgens in prenatal life and at puberty contribute to the masculinization of

human behavior. However, the behavioral significance of the transient activation of the hypothalamic–pituitary–gonadal (HPG) axis in early postnatal life remains largely unknown. Although early research on non-human primates indicated that suppression of the postnatal surge in testicular androgens had no measurable effects on the later expression of the male behavioral phenotype, recent research from our laboratory suggests that postnatal testosterone concentrations influence male infant preferences for larger social groups and temperament characteristics associated with the later development of aggression. In later assessment of gender-linked behavior in the second year of life, concentrations of testosterone at 3–4 months of age were unrelated to toy choices and activity levels during toy play. However, higher concentrations of testosterone predicted less vocalization in toddlers and higher parental ratings on an established screening measure for autism spectrum disorder. These findings suggest a role of the transient activation of the HPG axis in the development of typical and atypical male social relations and suggest that it may be useful in future research on the exaggerated rise in testosterone secretion in preterm infants or exposure to hormone disruptors in early postnatal life to include assessment of gender-relevant behavioral outcomes, including childhood disorders with sex-biased prevalence rates."

- Sex Differentiation: Organizing Effects of Sex Hormones
    - "Men and women differ, not only in their anatomy but also in their behavior. Research using animal models has convincingly shown that sex differences in the brain and behavior are induced by sex hormones during a specific, hormone-sensitive period during early development. Thus, a male-typical brain is organized under the influence of testosterone, mostly acting during fetal development, whereas a female-typical brain is organized under the influence of estradiol, mostly acting after birth, during a specific prepubertal period. Sex differences in behavior reflect sex differences in the brain, mostly in the hypothalamus and the olfactory system, the latter being important in mate selection. There is also evidence, albeit clinical, for a role of testosterone in the sexual differentiation of the human brain, in particular in inducing male gender role behavior and heterosexual orientation. However, whether estradiol is involved in the development of a female brain in humans still needs to be elucidated."
- 5α-reductase-2 Deficiency's Effect on Human Fertility
    - "A most interesting and intriguing male disorder of sexual differentiation is due to 5α-reductase-2 isoenzyme deficiency. These males are born with ambiguous external genitalia due to a deficiency in their ability to catalyze the conversion of testosterone to dihydrotestosterone (DHT). DHT is a potent androgen responsible for differentiation of the urogenital sinus and genital tubercle into the external genitalia, urethra and prostate. Affected males are born with a clitoral-like phallus, bifid scrotum, hypospadias, blind shallow vaginal pouch from incomplete closure of the urogenital sinus and a rudimentary prostate. At puberty, the surge in mainly testosterone production prompts virilization, causing most to choose gender reassignment to male.
      Fertility is a challenge for affected men for several reasons. Uncorrected cryptorchidism is associated with low sperm production, and there is evidence of defective transformation of spermatogonia into spermatocytes. The underdeveloped prostate and consequent low semen volumes affect sperm transport. Additionally, semen may not liquefy due to a lack of prostate-specific antigen. In this review, we discuss the 5α-reductase-2 deficiency syndrome and its impact on human fertility."

- Are there parental socialization effects on the sex-typed behavior of individuals with congenital adrenal hyperplasia?
    - "Influences of prenatal androgen exposure on human sex-typical behavior have been established largely through studies of individuals with congenital adrenal hyperplasia (CAH). However, evidence that addresses the potential confounding influence of parental socialization is limited. Parental socialization and its relationship to sex-typical toy play and spatial ability were investigated in two samples involving 137 individuals with CAH and 107 healthy controls. Females with CAH showed more boy-typical toy play and better targeting performance than control females, but did not differ in mental rotations performance. Males with CAH showed worse mental rotations performance than control males, but did not differ in sex-typical toy play or targeting. Reported parental encouragement of girl-typical toy play correlated with girl-typical toy play in all four groups. Moreover, parents reported encouraging less girl-typical, and more boy-typical, toy play in females with CAH than in control females and this reported encouragement partially mediated the relationship between CAH status and sex-typical toy play. Other evidence suggests that the reported parental encouragement of sex-atypical toy play in girls with CAH may be a response to the girls' preferences for boys' toys. Nevertheless, this encouragement could further increase boy-typical behavior in girls with CAH. In contrast to the results for toy play, we found no differential parental socialization for spatial activities and little evidence linking parental socialization to spatial ability. Overall, evidence suggests that prenatal androgen exposure and parental socialization both contribute to sex-typical toy play."
- Digit ratios (2D:4D), postnatal testosterone and eye contact in toddlers
    - "Previous research has shown an association between eye contact and prenatal testosterone measured in amniocenteses samples. The purpose of this study was to test the association between eye contact and prenatal androgen action measured via second to fourth digit ratios (2D:4D ratios), and to explore the relationship between eye contact and postnatal testosterone levels. Participants included 72 children, between the ages of 18 and 24 months, and their parents. Salivary testosterone levels were obtained when children were 3-months old. At 18-months, 2D:4D ratios were measured and parent-child dyads participated in an 8-min play session that was recorded and later coded for duration and frequency of eye contact. Results indicated that larger 2D:4D ratios (indicative of lower androgen levels) significantly predicted longer duration and more frequency of eye contact, while postnatal testosterone levels were unrelated to eye contact. These novel findings suggest prenatal androgens may influence the emergence of social development."
- From Gender Variance to Gender Dysphoria: Psychosexual development of gender atypical children and adolescents
    - "Children may show variability in their gender role behaviors, interests and preferences and/or their experienced gender identity (their experience to be male, female or a different gender). Within the male-female continuum of gender role expressions and gender identity three groups can be distin- guished. First, the *gender normative* children: Their gender role and gender identity are congruent with their natal sex. Second, the *gender variant* chil- dren: These children show (mild) cross-gender behaviors, interests and preferences, and may experience a gender identity which is congruent with their natal sex to a lesser extent than is the case in gender normative chil- dren. And third, the

gender dysphoric children: These children show extreme and enduring forms of cross-gender role expressions, experience a cross- gender identity and fulfill the criteria of a DSM-IV-TR diagnosis of Gender Identity Disorder (GID) (American Psychiatric Association 2000). In con- trast to most of the gender variant children, gender dysphoric children may need clinical attention as a result of significant distress or a significant risk of distress, and/or impairment in important areas of functioning. Knowledge about the future development, the trajectories and possible associated fac- tors of gender non-normative children (both gender variant and gender dysphoric) is however limited."

- Gender differences in neurodevelopment and epigenetics
    - "The concept that the brain differs in make-up between males and females is not new. For example, it is well established that anatomists in the nineteenth century found sex differences in human brain weight. The importance of sex differences in the organization of the brain cannot be overstated as they may directly affect cognitive functions, such as verbal skills and visuospatial tasks in a sex-dependent fashion. Moreover, the incidence of neurological and psychiatric diseases is also highly dependent on sex. These clinical observations reiterate the importance that gender must be taken into account as a relevant possible contributing factor in order to understand the pathogenesis of neurological and psychiatric disorders. Gender-dependent differentiation of the brain has been detected at every level of organization—morphological, neurochemical, and functional—and has been shown to be primarily controlled by sex differences in gonadal steroid hormone levels during perinatal development. In this review, we discuss how the gonadal steroid hormone testosterone and its metabolites affect downstream signaling cascades, including gonadal steroid receptor activation, and epigenetic events in order to differentiate the brain in a gender-dependent fashion."
- Postnatal testosterone levels and disorder relevant behavior in the second year of life
    - "The objective of the current study was to investigate the relationship between testosterone collected at 3-4 months of age and sex-linked disorder-relevant behaviors in the second year of life. Eighty-four children participated at 3-4 (when salivary testosterone levels were obtained and second to fourth digit ratios were measured) and 18-24 months of age (when behavioral ratings of aggression and verbal ability were coded from two 8-min play sessions). Parents also completed the Brief Infant-Toddler Social and Emotional Assessment, and the four subscales (Internalizing, Externalizing, Dysregulation, and Autism Spectrum Disorder) were used to indicate child specific problems. Greater postnatal testosterone levels in early infancy were predictive of more male-typical behaviors in the second year of life (i.e., more autism spectrum behaviors, less time vocalizing, and more Internalizing Problems). These results support the hypothesis that early infancy may be another critical period for the development of gender-linked behavior."
- Sexual Differentiation of the Human Brain in Relation to Gender-Identity, Sexual Orientation, and Neuropsychiatric Disorders
    - "During the intrauterine period, a testosterone surge in boys masculinizes the fetal brain, whereas the absence of such a surge in girls results in a feminine brain. Since sexual differentiation of the genitals takes place much earlier in intrauterine life than sexual differentiation of the human brain, these two processes can be influenced independently of each other. Gender identity (the conviction of belonging to the male or female gender), sexual orientation (hetero-, homo-, or bisexuality), pedophilia, and the risks for

neuropsychiatric disorders are programmed into our brain during early development. There is no proof that postnatal social environment has any crucial effect on gender identity or sexual orientation. We discuss the relationships between structural and functional sex differences of various brain areas and the way they change along with changes in the supply of sex hormones on the one hand and sex differences in behavior in health and disease on the other."

- The effects of prenatal sex steroid hormones on sexual differentiation of the brain
    - "Most of the anatomical, physiological and neurochemical gender-related differences in the brain occur prenatally. The sexual differences in the brain are affected by sex steroid hormones, which play important roles in the differentiation of neuroendocrine system and behavior. Testosterone, estrogen and dihydrotestosterone are the main steroid hormones responsible for the organization and sexual differentiation of brain structures during early development. The structural and behavioral differences in the female and male brains are observed in many animal species; however, these differences are variable between species. Animal and human (in vivo imaging and postmortem) studies on sex differences in the brain have shown many differences in the local distribution of the cortex, the gray-white matter ratio, corpus callosum, anterior commissure, hypothalamus, bed nucleus of the stria terminalis, limbic system and neurotransmitter systems. This review aims to evaluate the anatomical, physiological and neurochemical differences in the female and male brains and to assess the effect of prenatal exposure to sex steroid hormones on the developing brain."
- Early androgen effects on spatial and mechanical abilities: evidence from congenital adrenal hyperplasia
    - "There is considerable controversy about the origins of sex differences in cognitive abilities, particularly the male superiority in spatial abilities. We studied effects of early androgens on spatial and mechanical abilities in adolescents and young adults with congenital adrenal hyperplasia (CAH). On tests of three-dimensional mental rotations, geography, and mechanical knowledge, females with CAH scored higher than their unaffected sisters, and males with CAH scored lower than their unaffected brothers. Exploratory regression analyses suggest that androgens affect spatial ability in females directly and through male-typed activity interests. Findings indicate that early androgens influence spatial and mechanical abilities, and that androgen effects on abilities may occur in part through effects on sex-typed activity interests."
- PRIME PubMed | Early androgens, activity levels and toy choices of children in the second year of life
    - "The hypothesis that stronger preferences for active play styles contribute to stronger preferences for male-typical toys was examined in 47 boys and 37 girls at 19-months of age using ambulatory monitoring technology (i.e., actigraphy) to measure activity levels during contact with male-typical, female-typical, and gender-neutral toys. Digit ratios and salivary testosterone levels were measured earlier in children at 3-4 months of age. There were no significant sex differences in digit ratios, salivary testosterone levels, or overall activity levels during toy play. In contrast, contact times showed large sex differences in infants' toy preferences. The within-sex comparisons showed that infant girls had significant preferences for female-typical toys over male-typical toys, whereas infant boys showed only a small preference for male-typical toys over female-typical toys. More male-typical digit ratios in early infancy predicted higher activity counts during toy play

and less female-typical toy preferences in girls. However, in both sexes, activity levels were unrelated to toy preferences suggesting that factors other than activity level preferences contribute to the early emergence of gender-linked toy preferences."

- Fetal programming effects of testosterone on the reward system and behavioral approach tendencies in humans
    - *"Results:* Increasing FT predicted enhanced selectivity for positive compared with negatively valenced facial cues in reward-related regions such as caudate, putamen, and nucleus accumbens but not the amygdala. Statistical mediation analyses showed that increasing FT predicts increased behavioral approach tendencies by biasing caudate, putamen, and nucleus accumbens but not amygdala to be more responsive to positive compared with negatively valenced cues. In contrast, FT was not predictive of behavioral avoidance tendencies, either through direct or neurally mediated paths.
      Conclusions: This work suggests that testosterone in humans acts as a fetal programming mechanism on the reward system and influences behavioral approach tendencies later in life. As a mechanism influencing atypical development, FT might be important across a range of neuropsychiatric conditions that asymmetrically affect the sexes, the reward system, emotion processing, and approach behavior."
- Fetal Testosterone Influences Sexually Dimorphic Gray Matter in the Human Brain
    - "In nonhuman species, testosterone is known to have permanent organizing effects early in life that predict later expression of sex differences in brain and behavior. However, in humans, it is still unknown whether such mechanisms have organizing effects on neural sexual dimorphism. In human males, we show that variation in fetal testosterone (FT) predicts later local gray matter volume of specific brain regions in a direction that is congruent with sexual dimorphism observed in a large independent sample of age-matched males and females from the NIH Pediatric MRI Data Repository. Right temporoparietal junction/posterior superior temporal sulcus (RTPJ/pSTS), planum temporale/parietal operculum (PT/PO), and posterior lateral orbitofrontal cortex (plOFC) had local gray matter volume that was both sexually dimorphic and predicted in a congruent direction by FT. That is, gray matter volume in RTPJ/pSTS was greater for males compared to females and was positively predicted by FT. Conversely, gray matter volume in PT/PO and plOFC was greater in females compared to males and was negatively predicted by FT. Subregions of both amygdala and hypothalamus were also sexually dimorphic in the direction of Male > Female, but were not predicted by FT. However, FT positively predicted gray matter volume of a non-sexually dimorphic subregion of the amygdala. These results bridge a long-standing gap between human and nonhuman species by showing that FT acts as an organizing mechanism for the development of regional sexual dimorphism in the human brain."
- Pacific Center for Sex and Society - Intersex and Transsex: Atypical Gender Development and Social Construction
    - "In summary, the behaviors of intersexed and transgendered persons provide a wide range of evidence against many aspects of social science and social construction theory. Intersexed and transgendered persons, as well as typical persons, are each born with a certain background based upon evolutionary heritage, family genetics, uterine environment, and health factors that they will evidence in a socially permissive culture and limit in a restrictive one. The strongest gestational influences are from genetic and endocrinal organizing forces. Organizing factors are those genetic and hormonal

influences established prenatally that influence postnatal behaviors set in motion by social or other environmental activation processes (such as puberty) or events (such as serious threats). Organizing factors influence or bias subsequent responses of the individual to environmental/social forces; they predispose the person to manifest behaviors and attitudes (biases) that have come to be recognized as appropriate. Sex-related activation effects occur postnatally; most noticeably at or after puberty. The lives of intersex and transgendered persons provide strong evidence for a realistic theory of sexual development: biased-interaction theory."

- Relating Prenatal Testosterone Exposure to Postnatal Behavior in Typically Developing Children: Methods and Findings
    - "Testosterone levels during early development influence subsequent sex-typical behavior. These influences were initially identified in experimental research on nonhuman species. Additional research—primarily investigating individuals exposed to atypical hormone environments due to genetic disorders or maternal treatment with hormones during pregnancy—suggested that testosterone also influences the development of sex-typical behavior in humans. There is also interest in identifying relations between normal variability in the early hormone environment and normal variability in subsequent behavior. This article reviews studies that have assessed prenatal testosterone exposure in typically developing children using amniotic fluid sampling or maternal blood sampling. It concludes that both these approaches are promising, but both require larger samples than those used in most studies to date. Recommendations for future research also include using outcome measures that show sex differences, analyzing data within each sex, considering the time of day (as well as the time of gestation) when samples were taken, and reporting all the measures evaluated, not just those showing significant effects."
- Sex Steroids and the Organization of the Human Brain
    - "In humans, studies of the effects of FT often rely on indirect measures such as the ratio between the index finger (2D) and ring finger (4D) or on opposite-sex twin studies. Specifically, a smaller 2D:4D ratio correlates with higher FT exposure, and through the intrauterine presence of a male fetus, opposite-sex twin girls are ex- posed to higher FT levels than same-sex twin girls. Using the latter indirect mea- sure of FT, earlier reports showed that to- tal brain volume and cerebellum volume, typically found to be larger in males, were positively correlated with higher FT expo- sure (Peper et al., 2009)."
- Testosterone measured in infancy predicts subsequent sex-typed behavior in boys and in girls
    - "The testes are active during gestation, as well as during early infancy. Testosterone elevation during fetal development has been shown to play a role in human neurobehavioral sexual differentiation. The role of early postnatal gonadal activation in human psychosexual development is largely unknown, however. We measured testosterone in 48 full term infants (22 boys, 26 girls) by monthly urinary sampling from day 7 postnatal to age 6 months, and related the area under the curve (AUC) for testosterone during the first 6 months postnatal to subsequent sex-typed behavior, at the age of 14 months, using the Pre-School Activities Inventory (PSAI), and playroom observation of toy choices. In boys, testosterone AUC correlated significantly with PSAI scores (Spearman's rho = 0.54, p = 0.04). In addition, play with a train and with a baby doll showed the anticipated sex differences, and play with the train correlated significantly

and positively with testosterone AUC in girls (Spearman's rho = 0.43, p = 0.05), while play with the doll correlated significantly and negatively with testosterone AUC in boys (Spearman's rho = − 0.48, p < 0.03). These results may support a role for testosterone during early infancy in human neurobehavioral sexual differentiation."

- The relationship between second-to-fourth digit ratio and female gender identity
    - *"Results:* The 2D : 4D (mean ± standard deviation) in male, female, and GID-FtM were 0.945 ± 0.029, 0.999 ± 0.035, and 0.955 ± 0.029 in right hand and 0.941 ± 0.024, 0.979 ± 0.040, and 0.954 ± 0.036 in left hand, respectively. The 2D : 4D was significantly lower in male controls in both hands and GID-FtM in the right hand than in female controls (P < 0.05, analysis of variance). Multiple linear regression analysis revealed that "consistent gender identity" score in the higher domain in GIS and "persistent gender identity" score in the lower domain are statistically significant variables correlating with 2D : 4D in the right hands among biological females.
      Conclusions: The finger length ratio 2D : 4D in GID-FtM was significantly lower than in female controls in the right hand in this study. 2D : 4D showed a positive correlation with GIS score. Because 2D : 4D influences are assumed to be established in early life and to reflect testosterone exposure, our results suggest a relationship between GID-FtM and perinatal testosterone."
- Gender development and the human brain
    - "Convincing evidence indicates that prenatal exposure to the gonadal hormone, testosterone, influences the development of children's sex-typical toy and activity interests. In addition, growing evidence shows that testosterone exposure contributes similarly to the development of other human behaviors that show sex differences, including sexual orientation, core gender identity, and some, though not all, sex-related cognitive and personality characteristics. In addition to these prenatal hormonal influences, early infancy and puberty may provide additional critical periods when hormones influence human neurobehavioral organization. Sex-linked genes could also contribute to human gender development, and most sex-related characteristics are influenced by socialization and other aspects of postnatal experience, as well. Neural mechanisms underlying the influences of gonadal hormones on human behavior are beginning to be identified. Although the neural mechanisms underlying experiential influences remain largely uninvestigated, they could involve the same neural circuitry as that affected by hormones."
- Gendered Occupational Interests: Prenatal Androgen Effects on Psychological Orientation to Things Versus People
    - "There is considerable interest in understanding women's underrepresentation in science, technology, engineering, and mathematics careers. Career choices have been shown to be driven in part by interests, and gender differences in those interests have generally been considered to result from socialization. We explored the contribution of sex hormones to career-related interests, in particular studying whether prenatal androgens affect interests through psychological orientation to Things versus People. We examined this question in individuals with congenital adrenal hyperplasia (CAH), who have atypical exposure to androgens early in development, and their unaffected siblings (total *N* = 125 aged 9 to 26 years). Females with CAH had more interest in Things versus People than did unaffected females, and variations among females with CAH reflected variations in their degree of androgen exposure. Results provide strong support for hormonal

influences on interest in occupations characterized by working with Things versus People."

- Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior
    - "Both sexual orientation and sex-typical childhood behaviors, such as toy, playmate and activity preferences, show substantial sex differences, as well as substantial variability within each sex. In other species, behaviors that show sex differences are typically influenced by exposure to gonadal steroids, particularly testosterone and its metabolites, during early development (prenatally or neonatally). This article reviews the evidence regarding prenatal influences of gonadal steroids on human sexual orientation, as well as sex-typed childhood behaviors that predict subsequent sexual orientation. The evidence supports a role for prenatal testosterone exposure in the development of sex-typed interests in childhood, as well as in sexual orientation in later life, at least for some individuals. It appears, however, that other factors, in addition to hormones, play an important role in determining sexual orientation. These factors have not been well-characterized, but possibilities include direct genetic effects, and effects of maternal factors during pregnancy. Although a role for hormones during early development has been established, it also appears that there may be multiple pathways to a given sexual orientation outcome and some of these pathways may not involve hormones."
- Prenatal hormones and childhood sex segregation: playmate and play style preferences in girls with congenital adrenal hyperplasia
    - "We investigated playmate and play style preference in children with congenital adrenal hyperplasia (CAH) (26 females, 31 males) and their unaffected siblings (26 females, 17 males) using the Playmate and Play Style Preferences Structured Interview (PPPSI). Both unaffected boys and girls preferred same-sex playmates and sex-typical play styles. In the conflict condition where children chose between a same-sex playmate engaged in an other-sex activity or an other-sex playmate engaged in a same-sex activity, boys (both CAH and unaffected brothers) almost exclusively chose playmates based on the preferred play style of the playmate as opposed to the preferred gender label of the playmate. By contrast, unaffected girls used play style and gender label about equally when choosing playmates. Girls with CAH showed a pattern similar to that of boys: their playmate selections were more masculine than unaffected girls, they preferred a boy-typical play style and, in the conflict condition, chose playmates engaged in a masculine activity. These findings suggest that prenatal androgen exposure contributes to sex differences in playmate selection observed in typically developing children and that, among boys and girls exposed to high levels of androgens prenatally, play style preferences drive sex segregation in play."
- Sexual differentiation of human behavior: effects of prenatal and pubertal organizational hormones
    - "A key question concerns the extent to which sexual differentiation of human behavior is influenced by sex hormones present during sensitive periods of development (organizational effects), as occurs in other mammalian species. The most important sensitive period has been considered to be prenatal, but there is increasing attention to puberty as another organizational period, with the possibility of decreasing sensitivity to sex hormones across the pubertal transition. In this paper, we review evidence that sex hormones present during the prenatal and pubertal periods produce permanent changes

to behavior. There is good evidence that exposure to high levels of androgens during prenatal development results in masculinization of activity and occupational interests, sexual orientation, and some spatial abilities; prenatal androgens have a smaller effect on gender identity, and there is insufficient information about androgen effects on sex-linked behavior problems. There is little good evidence regarding long-lasting behavioral effects of pubertal hormones, but there is some suggestion that they influence gender identity and perhaps some sex-linked forms of psychopathology, and there are many opportunities to study this issue."

- Biological and psychosocial correlates of adult gender-variant identities: A review
    - "This article reviews research on biological and psychosocial factors relevant to the etiology of gender-variant identities. There is evidence for a genetic component of gender-variant identities through studies of twins and other within-family concordance and through studies of specific genes. Evidence that prenatal androgens play a role comes from studies that have examined finger length ratios (2D:4D), prevalence of polycystic ovary syndrome among female-to-male transsexuals, and individuals with intersex and related conditions who are more likely to have reassigned genders. There is also evidence that transsexuals have parts of their brain structure that is typical of the opposite birth-assigned gender. A greater likelihood of non-right-handedness suggests developmental instability may also contribute as a biological factor. There is a greater tendency for persons with gender-variant identities to report childhood abuse and a poor or absent relationship with parents. It is unclear if this is a cause or effect of a gender-variant identity. Parental encouragement of gender-variance is more common among individuals who later develop a gender-variant identity. We conclude that biological factors, especially prenatal androgen levels, play a role in the development of a gender-variant identity and it is likely that psychosocial variables play a role in interaction with these factors."
- Mentale Rotation bei Mann-zu-Frau-Transsexuellen und Männern ohne Geschlechtsidentitätsstörung ("Mental rotation in male-to-female transsexuals and men without gender identity disorder")
    - "This work was able to show that there were differences in cortical activation even when comparing transsexual men before HRT and men without GID. M-F transsexuals before HRT mainly activated frontal and occipitotemporal areas more than men without GID, while men without GIS showed more activation in the inferior parietal lobe of the left hemisphere compared to M-F transsexuals before HRT. There were clear parallels between the activation differences and the known activation differences between men and women without GID (Butler et al., 2006; Gizewski et al., 2006). These observations provide indications that prenatal hormonal fluctuations may be a component of the multifactorial imprinting of sexually dimorphic cortical functions and the development of transsexuality."
- Psychosexual Development in Children with Disorder of Sex Development (DSD) – Results from the German Clinical Evaluation Study
    - "Psychosexual development is influenced by biological and psychosocial factors. Human beings show a great variability in psychosexual development both between and within gender-groups. However, there are relatively stable gender-related behaviors and self-perceptions, in which males and females differ distinctly. There is strong evidence that high concentrations of androgens lead to more male-typical behavior and that this

also influences gender identity. Disorders of sex development (DSD) provide the opportunity to analyze the role of different factors on psychosexual development. We examined 166 children age 4 to 12 with DSD using instruments concerning gender role behavior, gender identity, and friendship. Results underline the hypothesis, that androgens play a decisive role in the masculinization of gender role behavior in children. There are also some relations between the experiences of gender change and psychosexual outcomes which have to be discussed. Nevertheless, results indicated a high congruence between the children's gender identity and gender of rearing."

- Sexual differentiation of the brain related to gender identity: beyond hormones
    - "The sexual differentiation of the brain starts in the second semester of pregnancy, which is, after the development of the genitals which differentiate in the second month of pregnancy. Because these two processes have different timetables, it could be that these are initiated through different pathways. Male gonads synthesize testosterone, which can be converted into estrogen by aromatase in the brain. In humans, the exact mechanism of male and female brain development has still to be elucidated. Based on clinical evidence from genetic men (XY) suffering from a mutation in the androgen receptor gene (complete androgen-insensitivity syndrome) and who show a female phenotype of the external genitals as well as the brain, it can be proposed that direct action of testosterone is probably causing the brain to differentiate in the male direction. However, when the process of genital development and of brain sexual development does not match the same sex, females with a male brain and vice versa can arise. These transsexual people have problems with their gender identity and have the conviction of being born in the wrong body. Twin and family studies show that there are genetic factors influencing the chances of a gender identity problem. Genetic factors could play a large role in the sexual differentiation of the brain, as can be shown from studies where differential genetic expression is found before development of the gonads. These genes could also function in other tissues than gonads and influence the sexual differentiation of the brain. The DMRT gene family which encodes transcription factors or the amount of sex hormone binding globulin (SHBG) is possibly influencing the development of sex differences, just as sex-biased differential splicing. Epigenetic mechanisms such as X-inactivation and genomic imprinting are also good candidates for causing differences in the sexual differentiation of the brain. These observations indicate that probably many processes operate together in the sexual differentiation of the brain and that diverse mutations can lead to gender identity problems."
- Sexual Hormones and the Brain: An Essential Alliance for Sexual Identity and Sexual Orientation
    - "The fetal brain develops during the intrauterine period in the male direction through a direct action of testosterone on the developing nerve cells, or in the female direction through the absence of this hormone surge. In this way, our gender identity (the conviction of belonging to the male or female gender) and sexual orientation are programmed or organized into our brain structures when we are still in the womb. However, since sexual differentiation of the genitals takes place in the first two months of pregnancy and sexual differentiation of the brain starts in the second half of pregnancy, these two processes can be influenced independently, which may result in extreme cases in trans-sexuality. This also means that in the event of ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of

masculinization of the brain. There is no indication that social environment after birth has an effect on gender identity or sexual orientation."

- Pacific Center for Sex and Society - Clinical Implications of the Organizational and Activational Effects of Hormones
    - "Debate on the relative contributions of nature and nurture to an individual's gender patterns, sexual orientation and gender identity are reviewed as they appeared to this observer starting from the middle of the last century. Particular attention is given to the organization-activation theory in comparison to what might be called a theory of psychosexual neutrality at birth or rearing consistency theory. The organization-activation theory posits that the nervous system of a developing fetus responds to prenatal androgens so that, at a postnatal time, it will determine how sexual behavior is manifested. How organization-activation was or was not considered among different groups and under which circumstances it is considered is basically understood from the research and comments of different investigators and clinicians. The preponderance of evidence seems to indicate that the theory of organization-activation for the development of sexual behavior is certain for non-human mammals and almost certain for humans. This article also follows up on previous clinical critiques and recommendations and makes some new suggestions."
- Disorders of sex development expose transcriptional autonomy of genetic sex and androgen-programmed hormonal sex in human blood leukocytes
    - "*Results:* A discrete set of transcripts was directly correlated with XY or XX genotypes in all individuals independent of male or female phenotype of the external genitalia. However, a significantly larger gene set in the PBMC only reflected the degree of external genital masculinization independent of the sex chromosomes and independent of concurrent post-natal sex steroid hormone levels. Consequently, the architecture of the transcriptional PBMC-"sexes" was either male, female or even "intersex" with a discordant alignment of the DSD individuals' genetic and hormonal sex signatures.
      Conclusions: A significant fraction of gene expression differences between males and females in the human appears to have its roots in early embryogenesis and is not only caused by sex chromosomes but also by long-term sex-specific hormonal programming due to presence or absence of androgen during the time of external genital masculinization. Genetic sex and the androgen milieu during embryonic development might therefore independently modulate functional traits, phenotype and diseases associated with male or female gender as well as with DSD conditions."
- Fetal testosterone predicts sexually differentiated childhood behavior in girls and in boys
    - "Mammals, including humans, show sex differences in juvenile play behavior. In rodents and nonhuman primates, these behavioral sex differences result, in part, from sex differences in androgens during early development. Girls exposed to high levels of androgen prenatally, because of the genetic disorder congenital adrenal hyperplasia, show increased male-typical play, suggesting similar hormonal influences on human development, at least in females. Here, we report that fetal testosterone measured from amniotic fluid relates positively to male-typical scores on a standardized questionnaire measure of sex-typical play in both boys and girls. These results show, for the first time, a link between fetal testosterone and the development of sex-typical play in children from the general population, and are the first data linking high levels of prenatal testosterone to increased male-typical play behavior in boys."

- Hormone-behavior associations in early infancy
    - "The physiological significance of hormonal changes in early postnatal life is emerging, but the behavioral significance in humans is unknown. As a first test of the relationship between hormones and behavior in early infancy we measured digit ratios and salivary hormone levels in forty-one male and female infants (3-4 months of age) who watched a video depicting stimuli differentially preferred by older males and females (toys, groups). An eye-tracker measured visual fixations and looking times. In female infants, hormones were unrelated to visual preferences. In male infants, higher androgen levels predicted stronger preferences for male-typical stimuli. These data provide the first evidence for a role for hormones in emerging sex-linked behavior in early development."
- Sex differences and the impact of steroid hormones on the developing human brain
    - "Little is known about the hormonal effects of puberty on the anatomy of the developing human brain. In a voxel-based morphometry study, sex-related differences in gray matter (GM) volume were examined in 46 subjects aged 8-15 years. Males had larger GM volumes in the left amygdala, whereas females had larger right striatal and bilateral hippocampal GM volumes than males. Sexually dimorphic areas were related to Tanner stages (TS) of pubertal development and to circulating level of steroid hormones in a subsample of 30 subjects. Regardless of sex, amygdala and hippocampal volumes varied as a function of TS and were associated with circulating testosterone (TEST) levels. By contrast, striatal GM volumes were unrelated to pubertal development and circulating steroid hormones. Whole-brain regression analyses revealed positive associations between circulating estrogen levels and parahippocampal GM volumes as well as between TEST levels and diencephalic brain structures. In addition, a negative association was found between circulating TEST and left parietal GM volumes. These data suggest that GM development in certain brain regions is associated with sexual maturation and that pubertal hormones might have organizational effects on the developing human brain."
- Prenatal exposure to sex steroid hormones and behavioral/cognitive outcomes
    - "Experimental studies in animals indicate that androgen exposure in fetal or neonatal life largely accounts for known sex differences in brain structure and behavior. Clinical research in humans suggests similar influences of early androgen concentrations on some behaviors that show sex differences, including play behavior in childhood and sexual orientation in adulthood. Available research also suggests that sex steroid hormone exposure may contribute to sex differences in the risk of autism and affective disorders in schizophrenia. However, findings have been inconsistent for other characteristics that show sex differences, including aggression and spatial ability. Moreover, social and environmental factors may modulate some of the associations observed. This article reviews the evidence that early-life exposure to sex steroid hormones contributes to sexually dimorphic behavior and cognitive abilities in humans."
- Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess
    - "46,XX individuals with classical congenital adrenal hyperplasia (CAH) due to deficiency of the enzyme, 21-hydroxylase, show variable degrees of masculinization of body and behavior due to excess adrenal androgen production. Increased bisexuality and homosexuality have also been reported. This article provides a review of existing reports of the latter and presents a new study aimed at replicating the previous findings with

detailed assessments of sexual orientation on relatively large samples, and at extending the investigation to the mildest form, non-classical (NC) CAH. Also, this is the first study to relate sexual orientation to the specific molecular genotypes of CAH. In the present study, 40 salt-wasters (SW), 21 SV (simple-virilizing), 82 NC, and 24 non-CAH control women (sisters and female cousins of CAH women) were blindly administered the Sexual Behavior Assessment Schedule (SEBAS-A, 1983 ed.; H. F. L. Meyer-Bahlburg & A. A. Ehrhardt, Privately printed). Most women were heterosexual, but the rates of bisexual and homosexual orientation were increased above controls not only in women with classical CAH, but also in NC women, and correlated with the degree of prenatal androgenization. Classifying women by molecular genotypes did not further increase the correlation. Diverse aspects of sexual orientation were highly intercorrelated, and principal components analysis yielded one general factor. Bisexual/homosexual orientation was (modestly) correlated with global measures of masculinization of non-sexual behavior and predicted independently by the degree of both prenatal androgenization and masculinization of childhood behavior. We conclude that the findings support a sexual-differentiation perspective involving prenatal androgens on the development of sexual orientation."

- Androgens and eye movements in women and men during a test of mental rotation ability
    - "Eye movements were monitored in 16 women and 20 men during completion of a standard diagram-based test of mental rotation ability to provide measures of cognitive function not requiring conscious, decisional processes. Overall, women and men allocated visual attention during task performance in very similar, systematic ways. However, consistent with previous suggestions that sex differences in attentional processes during completion of the mental rotation task may exist, eye movements in men compared to women indicated greater discrimination and longer processing of correct alternatives during task performance. Other findings suggested that androgens may enhance cognitive processes that are recruited differentially by women and men as a function of the task. Specifically, smaller (i.e., more masculine) digit ratios were associated with men's shorter fixations on distracters, suggesting that perinatal androgen action may influence brain systems that facilitate the identification of relevant task stimuli. In women, higher circulating testosterone levels appeared to contribute to more general processes engaged during task performance, for example higher levels of visual persistence. It is possible that variability in the relative contribution of such hormone sensitive cognitive processes to accuracy scores as a function of different sample characteristics or assessment methods may partially account for the inconsistent findings of previous research on hormonal factors in mental rotation ability."
- Sexual differentiation of the brain and behavior
    - "During the intrauterine period the human brain develops in the male direction via direct action of a boy's testosterone, and in the female direction through the absence of this hormone in a girl. During this time, gender identity (the feeling of being a man or a woman), sexual orientation, and other behaviors are programmed. As sexual differentiation of the genitals takes places in the first 2 months of pregnancy, and sexual differentiation of the brain starts during the second half of pregnancy, these two processes may be influenced independently of each other, resulting in transsexuality. This also means that in the case of an ambiguous gender at birth, the degree of

masculinization of the genitals may not reflect the same degree of masculinization of the brain. Differences in brain structures and brain functions have been found that are related to sexual orientation and gender."

- The control of sexual differentiation of the reproductive system and brain
    - "This review summarizes current knowledge of the genetic and hormonal control of sexual differentiation of the reproductive system, brain and brain function. While the chromosomal regulation of sexual differentiation has been understood for over 60 years, the genes involved and their actions on the reproductive system and brain are still under investigation. In 1990, the predicted testicular determining factor was shown to be the SRY gene. However, this discovery has not been followed up by elucidation of the actions of SRY, which may either stimulate a cascade of downstream genes, or inhibit a suppressor gene. The number of other genes known to be involved in sexual differentiation is increasing and the way in which they may interact is discussed. The hormonal control of sexual differentiation is well-established in rodents, in which prenatal androgens masculinize the reproductive tract and perinatal oestradiol (derived from testosterone) masculinizes the brain. In humans, genetic mutations have revealed that it is probably prenatal testosterone that masculinizes both the reproductive system and the brain. Sexual differentiation of brain structures and the way in which steroids induce this differentiation, is an active research area. The multiplicity of steroid actions, which may be specific to individual cell types, demonstrates how a single hormonal regulator, e.g. oestradiol, can exert different and even opposite actions at different sites. This complexity is enhanced by the involvement of neurotransmitters as mediators of steroid hormone actions. In view of current environmental concerns, a brief summary of the effects of endocrine disruptors on sexual differentiation is presented."
- Associations among gender-linked toy preferences, spatial ability, and digit ratio: evidence from eye-tracking analysis
    - "Eye-tracking technology was used to monitor eye-movements in 64 adults (age range, 18-22 years) during simultaneous presentation of "masculine" and "feminine" toys. Women and men who showed more visual fixations on male-typical toys compared to female-typical toys had significantly better targeting ability and smaller (i.e., more masculine) digit ratios, a putative marker of prenatal androgen levels. In contrast, individuals with visual preferences for female-typical or male-typical toys did not differ in mental rotations ability and in their retrospective reports of childhood gender-linked activities. The finding that targeting ability and digit ratios varied according to visual preferences for gender-linked toys suggests that prenatal androgens promote enduring preferences for male-typical objects and indicate further that some gender-linked traits vary according to the direction of a visual preference for gender-linked toys. Visual preferences derived from eye-tracking, therefore, may be a useful supplement to current measures of psychosexual differentiation in hormone-behavior research, particularly because eye-movements are not dependent on verbal abilities or subjective evaluations of behavior."
- Prenatal testosterone and gender-related behaviour
    - "Testosterone plays an important role in mammalian brain development. In neural regions with appropriate receptors testosterone, or its metabolites, influences patterns of cell death and survival, neural connectivity and neurochemical characterization. Consequently, testosterone exposure during critical periods of early development

produces permanent behavioural changes. In humans, affected behaviours include childhood play behaviour, sexual orientation, core gender identity and other characteristics that show sex differences (i.e. differ on average between males and females). These influences have been demonstrated primarily in individuals who experienced marked prenatal hormone abnormalities and associated ambiguities of genital development (e.g. congenital adrenal hyperplasia). However, there is also evidence that testosterone works within the normal range to make some individuals within each sex more sex-typical than others. The size of testosterone-related influences, and perhaps even their existence, varies from one sex-typed characteristic to another. For instance: prenatal exposure to high levels of testosterone has a substantial influence on sex-typical play behaviour, including sex-typed toy preferences, whereas influences on core gender identify and sexual orientation are less dramatic. In addition: there appears to be little or no influence of prenatal testosterone on mental rotations ability, although mental rotations ability shows a marked sex difference. These findings have implications for basic understanding of the role of testosterone in normative gender development, as well as for the clinical management of individuals with disorders of sex development (formerly called intersex syndromes)."

- Topical Review: Fetal Testosterone and Sex Differences in Typical Social Development and in Autism - Rebecca Christine Knickmeyer, Simon Baron-Cohen, 2006
    - "Experiments in animals leave no doubt that androgens, including testosterone, produced by the testes in fetal and/or neonatal life act on the brain to induce sex differences in neural structure and function. In human beings, there is evidence supporting a female superiority in the ability to read nonverbal signals, specific language-related skills, and theory of mind. Even more striking than the sex differences seen in the typical population is the elevated occurrence of social and communicative difficulties in human males. One such condition, autism, occurs four times more frequently in boys than in girls. Recently, a novel theory known as the ``extreme male brain'' has been proposed. It suggests that the behaviors seen in autism are an exaggeration of typical sex differences and that exposure to high levels of prenatal testosterone might be a risk factor. In this article, we argue that prenatal and neonatal testosterone exposures are strong candidates for having a causal role in sexual dimorphism in human behavior, including social development, and as risk factors for conditions characterized by social impairments, particularly autism spectrum conditions."
- Prenatal exposure to diethylstilbestrol (DES) in males and gender-related disorders: Results from a 5-year study
    - "For many years, researchers and public health specialists have been assessing the human health impact of prenatal exposure to the estrogenic anti-miscarriage drug, diethylstilbestrol (commonly known as DES or "stilbestrol"). The scope of adverse effects in females exposed to DES (often called "DES daughters") has been more substantially documented than the effects in males ("DES sons"). This paper contributes three areas of important research on DES exposure in males: (1) an overview of published literature discussing the confirmed and suspected adverse effects of prenatal exposure in DES sons; (2) preliminary results from a 5-year online study of DES sons involving 500 individuals with confirmed (60% of sample) and suspected prenatal DES exposure; (3) documentation of the presence of gender identity disorders and male-to-female transsexualism reported by more than 100 participants in the study."

- Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings
    - "There is now good evidence that human sex-typed behavior is influenced by sex hormones that are present during prenatal development, confirming studies in other mammalian species. Most of the evidence comes from clinical populations, in which prenatal hormone exposure is atypical for a person's sex, but there is increasing evidence from the normal population for the importance of prenatal hormones. In this paper, we briefly review the evidence, focusing attention on the methods used to study behavioral effects of prenatal hormones. We discuss the promises and pitfalls of various types of studies, including those using clinical populations (concentrating on those most commonly studied, congenital adrenal hyperplasia, androgen insensitivity syndrome, ablatio penis, and cloacal exstrophy), direct measures of hormones in the general population (assayed through umbilical cord blood, amniotic fluid, and maternal serum during pregnancy), and indirect measures of hormones in the general population (inferred from intrauterine position and biomarkers such as otoacoustic emissions, finger length ratios, and dermatoglyphic asymmetries). We conclude with suggestions for interpreting and conducting studies of the behavioral effects of prenatal hormones."
- Pubertal hormones organize the adolescent brain and behavior
    - "Maturation of the reproductive system during puberty results in elevated levels of gonadal steroid hormones. These hormones sculpt neural circuits during adolescence, a time of dramatic rewiring of the nervous system. Here, we review the evidence that steroid-dependent organization of the adolescent brain programs a variety of adult behaviors in animals and humans. Converging lines of evidence indicate that adolescence may be a sensitive period for steroid-dependent brain organization and that variation in the timing of interactions between the hormones of puberty and the adolescent brain leads to individual differences in adult behavior and risk of sex-biased psychopathologies."
- PRIME PubMed | Androgen and psychosexual development: core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH)
    - "We assessed core gender identity, sexual orientation, and recalled childhood gender role behavior in 16 women and 9 men with CAH and in 15 unaffected female and 10 unaffected male relatives, all between the ages of 18 and 44 years. Women with congenital adrenal hyperplasia (CAH) recalled significantly more male-typical play behavior as children than did unaffected women, whereas men with and without CAH did not differ. Women with CAH also reported significantly less satisfaction with the female sex of assignment and less heterosexual interest than did unaffected women. Again, men with CAH did not differ significantly from unaffected men in these respects. Our results for women with CAH are consistent with numerous prior reports indicating that girls with CAH show increased male-typical play behavior. They also support the hypotheses that these women show reduced heterosexual interest and reduced satisfaction with the female sex of assignment. Our results for males are consistent with most prior reports that boys with CAH do not show a general alteration in childhood play behavior. In addition, they provide initial evidence that core gender identity and sexual orientation are unaffected in men with CAH. Finally, among women with CAH, we found that recalled male-typical play in childhood correlated with reduced satisfaction with the female

gender and reduced heterosexual interest in adulthood. Although prospective studies are needed, these results suggest that those girls with CAH who show the greatest alterations in childhood play behavior may be the most likely to develop a bisexual or homosexual orientation as adults and to be dissatisfied with the female sex of assignment."

- Environment- and gene-dependent human ontogenesis, sociogenesis and phylogenesis (eco-geno-onto-socio-phylogenesis)
    - "Prevention of environment- and gene-dependent, teratogenic malfunctions ("Functional Teratogenesis")-- caused by abnormal hormone, neurotransmitter and cytokine concentrations during organization of the neuro-endocrine-immune system (NEIS) should be considered as a global challenge of outstanding relevance. By optimizing the natural and social environment and correcting in time abnormal concentrations of hormones, neurotransmitters and cytokines during the critical perinatal (pre- and early postnatal) organization period of the NEIS ("Neuro-Endocrine-Immune Prophylaxis") human ontogenesis and sociogenesis can be decisively improved ("Primary Prevention of Maldevelopments of Human Beings and their Societies"). Finally, phylogenesis is dependent on incessant sequencies of ontogenesis and sociogenesis ("Onto-Socio-Phylogenesis")."
- Gender role across development in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency
    - "This study evaluated the degree of femininity and masculinity at different developmental stages in a group of adult women, some of whom were exposed to elevated prenatal adrenal androgens as a result of congenital adrenal hyperplasia (CAH) due to 21 hydroxylase (21-OH) deficiency. Women who had presented to the Johns Hopkins Hospital Pediatric Endocrine Clinic for treatment of CAH due to 21-OH deficiency were included. The control group consisted of sisters of CAH participants and women referred for evaluation of polycystic ovary syndrome. Study participants were given a questionnaire asking them to indicate their degree of masculinity and femininity during childhood, adolescence, and adulthood. In addition, participants were asked questions related to their play behavior during childhood, including playmate preferences, toy preferences, and admiration of male or female characters during fantasy play. Across participant groups, self-reported femininity decreased in a dose response manner, according to prenatal androgen exposure. For all groups, femininity increased through developmental stages. Women with salt-losing CAH remained less feminine than controls into adulthood. Conversely, self-reported masculinity increased in a dose-response manner, according to prenatal androgen exposure, across participant groups. Women with CAH showed a decrease in masculinity across developmental stages, such that by adulthood, there were no significant differences in masculinity between controls and the women with CAH. Women with salt-losing CAH were more likely to recall preferences for boy playmates, male-typical toys, and admiration for male characters during childhood than other study participants. Our data support the effect of both prenatal androgen exposure and socialization on gender role behavior in adult women with CAH due to 21-OH deficiency."
- Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls

    - "In animals it has been shown that exposure to sex hormones is influenced by intrauterine position. Thus fetuses located between two male fetuses are exposed to higher levels of testosterone (T) than fetuses situated between two female fetuses or one female and one male fetus. In a group of opposite-sex (OS) twin girls and same-sex (SS) twin girls a potential effect of prenatal exposure to testosterone (T) on functional cerebral lateralization was investigated. We hypothesized that prenatal exposure to T would result in a more masculine, i.e. a more lateralized pattern of cerebral lateralization in OS twin girls than in SS twin girls. An auditory-verbal dichotic listening task (DLT) was used as an indirect method to study hemispheric specialization. Firstly, we established a sex difference on the DLT. Compared with SS girls, OS twin boys showed a more lateralized pattern of processing verbal stimuli. Secondly, as predicted OS girls had a more masculine pattern of cerebral lateralization, than SS girls. These findings support the notion of an influence of prenatal T on early brain organization in girls."
- Sexual differentiation of the human brain: relevance for gender identity, transsexualism and sexual orientation
    - "Male sexual differentiation of the brain and behavior are thought, on the basis of experiments in rodents, to be caused by androgens, following conversion to estrogens. However, observations in human subjects with genetic and other disorders show that direct effects of testosterone on the developing fetal brain are of major importance for the development of male gender identity and male heterosexual orientation. Solid evidence for the importance of postnatal social factors is lacking. In the human brain, structural differences have been described that seem to be related to gender identity and sexual orientation."
- Sex in the brain. Gender differences in the human hypothalamus and adjacent areas. Relationship to transsexualism, sexual orientation, sex hormone receptors and endocrine status.
    - "From the moment of conception until the moment we die we are living in a sex-differentiated world. Not only do men and women have different physiques, there are also sex differences in seeing, smelling, thinking, feeling and behaving (Savic et al., 2001; Rahman and Wilson, 2002; Collaer and Nelson, 2002; Goorenn and Kruijver, 2002; Malcolm et al., 2002; Loring-Meier and Halpern, 2002; Karama et al., 2002; Canli et al., 2002; Fischer et al., 2004; Hamann et al., 2004). Also in terms of life expectancy and the risk to develop body and brain related diseases as well as in the way men and women respond to medical treatments, both sexes are known to differ from each other (MacLusky and Naftolin, 1981; Swaab and Hofman, 1995; Collaer and Hines, 1995; Swaab, 2002). "
- PRIME PubMed | Prenatal androgens and gender-typed behavior: a study of girls with mild and severe forms of congenital adrenal hyperplasia
    - "Gender-typed behaviors and interests were investigated in 26 girls, aged 2-10 years, affected with congenital adrenal hyperplasia (CAH) and in 26 unaffected girls matched for age. Girls with CAH were more interested in masculine toys and less interested in feminine toys and were more likely to report having male playmates and to wish for masculine careers. Parents of girls with CAH rated their daughters' behaviors as more boylike than did parents of unaffected girls. A relation was found between disease severity and behavior indicating that more severely affected CAH girls were more interested in masculine toys and careers. No parental influence could be demonstrated

on play behavior, nor did the comparison of parents' ratings of wished for behavior versus perceived behavior in their daughters indicate an effect of parental expectations. The results are interpreted as supporting a biological contribution to differences in play behavior between girls with and without CAH."

- Sex steroids and human behavior: prenatal androgen exposure and sex-typical play behavior in children
    - "Gonadal hormones, particularly androgens, direct certain aspects of brain development and exert permanent influences on sex-typical behavior in nonhuman mammals. Androgens also influence human behavioral development, with the most convincing evidence coming from studies of sex-typical play. Girls exposed to unusually high levels of androgens prenatally, because they have the genetic disorder, congenital adrenal hyperplasia (CAH), show increased preferences for toys and activities usually preferred by boys, and for male playmates, and decreased preferences for toys and activities usually preferred by girls. Normal variability in androgen prenatally also has been related to subsequent sex-typed play behavior in girls, and nonhuman primates have been observed to show sex-typed preferences for human toys. These findings suggest that androgen during early development influences childhood play behavior in humans at least in part by altering brain development."
- Androgen imprinting of the brain in animal models and humans with intersex disorders: review and recommendations
    - "Animal studies support a role for postnatal androgens in brain/behavior development with human studies neither completely supportive nor antagonistic. Therefore, gender assignment in infants with intersex should be made with the possibility in mind that postnatal testicular hormones at ages 1 to 6 months may affect gender identity. A case-control study is required to test the hypothesis that postnatal androgen exposure may convert ambisexual brain functions to committed male behavior patterns."
- Organizing and activating effects of sex hormones in homosexual transsexuals
    - "The cause of transsexualism remains unclear. The hypothesis that atypical prenatal hormone exposure could be a factor in the development of transsexualism was examined by establishing whether an atypical pattern of cognitive functioning was present in homosexual transsexuals. Possible activating effects of sex hormones as a result of cross-sex hormone treatment were also studied. Female-to-male and male-to-female transsexuals were compared with female and male controls with respect to spatial ability before and after treatment. The data were consistent with an organizing effect, but there was no evidence of an activating effect. Homosexual transsexuals, who prior to hormone treatment scored in the direction of the opposite sex, may have reached a ceiling in performance and therefore do not benefit from activating hormonal effects."
- Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure assessed by CYP21 genotype in girls with congenital adrenal hyperplasia
    - "Previous studies have shown that girls with congenital adrenal hyperplasia (CAH), a syndrome resulting in overproduction of adrenal androgens from early fetal life, are behaviorally masculinized. We studied play with toys in a structured play situation and correlated the results with disease severity, assessed by CYP21 genotyping, and age at diagnosis. Girls with CAH played more with masculine toys than controls when playing alone. In addition, we could demonstrate a dose-response relationship between disease severity (i.e. degree of fetal androgen exposure) and degree of masculinization of

behavior. The presence of a parent did not influence the CAH girls to play in a more masculine fashion. Four CAH girls with late diagnosis are also described. Three of the four girls played exclusively with one of the masculine toys, a constructional toy. Our results support the view that prenatal androgen exposure has a direct organizational effect on the human brain to determine certain aspects of sex-typed behavior."

- Genetic and epigenetic effects on sexual brain organization mediated by sex hormones
    - "Alterations of sex hormone levels during pre- or perinatal sexual brain organization - responsible for long-term changes of gonadotropin secretion, sexual orientation, and gender role behavior - can be caused by: 1. Genetic effects, i.e. mutations or polymorphisms of a) 21-hydroxylase genes on chromosome 6, b) 3beta-hydroxysteroid dehydrogenase genes in chromosome 1 or c) X-chromosomal genes, and 2. Epigenetic effects, such as a) stressful situations - especially in combination with mutations - and b) endocrine disrupters, e.g. the pesticide DDT and its metabolites, which display estrogenic, antiandrogenic, and inhibitory effects on the enzyme 3beta-hydroxysteroid dehydrogenase leading to increased levels of dehydroepiandrosterone and its sulfate as precursors of endogenous androgens and estrogens. In connection with the introduction and extensive use of the pesticide DDT, the following findings were obtained in subjects born before as compared to those born during this period: 1. The prevalence of patients with polycystic ovaries (PCO), idiopatic oligospermia (IO), and transsexualism (TS) increased significantly (about 3-4 fold). 2. Partial 21-hydroxylase deficiencies were observed in most patients with PCO and TS and some patients with IO born before this period. 3. In contrast, most patients with PCO and TS and several patients with IO born during the period of massive use of DDT displayed clearly increased plasma levels of dehydroepiandrosterone sulfate (DHEA-S) and DHEA-S/cortisol ratios suggesting partial 3beta-hydroxsteroid dehydrogenase (3beta-HSD) deficiencies. Interestingly enough, geneticists could not find any mutations of 3beta-HSD genes in such subjects. However, o,p'-DDT and/or its metabolite o,p'-DDD are strong inhibitors of 3beta-HSD, indicating their possible co-responsibility for such life-long ontogenetic alterations. Finally, some data suggest that endocrine disrupters may also be able to affect the development of sexual orientation."
- Biological limits of gender construction.
    - "A biosocial theory of gender is constructed on both the macro and micro levels. A micro-model of within-sex differences among females integrates the biological model current in primatology with the prevailing social science model. It shows how sex differences in hormone experience from gestation to adulthood shape gendered behavior (that is, behavior that differs by sex). On the macro level, this model also illustrates how socialization and environment shape gendered behavior. It then demonstrates how hormone experiences can facilitate or dampen the effects of socialization and environment on gendered behavior. Data were analyzed from a sample of 163 White women who were studied from before they were born to the end of their 3rd decade. Results show that prenatal androgen exposures from the 2nd trimester affected gendered behavior, but not exposures from the 1st or 3rd trimesters. Further, the basic hormone model shows that in this sample, mothers' prenatal hormones had an effect on the gendered behavior of the Ss 3 decades later. The author speculates about the constraints placed by biology on the social reconstruction of gender."
- Sex differences in the distribution of androgen receptors in the human hypothalamus

    - "The present study reports for the first time the distribution of androgen receptor immunoreactivity (AR-ir) in the human hypothalamus of ten human subjects (five men and five women) ranging in age between 20 years and 39 years using the antibody PG21. Prolonged postmortem delay (72:00 hours) or fixation time (100 days) did not influence the AR-ir. In men, intense nuclear AR-ir was found in neurons of the horizontal limb of the diagonal band of Broca, in neurons of the lateromamillary nucleus (LMN), and in the medial mamillary nucleus (MMN). An intermediate nuclear staining was found in the diagonal band of Broca, sexually dimorphic nucleus of the preoptic area, paraventricular nucleus, suprachiasmatic nucleus, ventromedial nucleus, and infundibular nucleus, whereas weaker labeling was found in the bed nucleus of the stria terminalis, medial preoptic area, dorsal and ventral zones of the periventricular nucleus, supraoptic nucleus, and nucleus basalis of Meynert. In most brain areas, women revealed less staining than men. In the LMN and the MMN, a strong sex difference was found. Cytoplasmic labeling was observed in neurons of both sexes, although women showed a higher variability in the intensity of such staining. However, no sex differences in AR-ir were observed in the bed nucleus of the stria terminalis, the nucleus basalis of Meynert, or the islands of Calleja. Species differences and similarities of the AR-ir distribution are discussed. The present results suggest the participation of androgens in the regulation of various hypothalamic processes that are sexually dimorphic."
- The development of brain sex differences: a multisignaling process
    - "In order to account for the development of sex differences in the brain, we took, as an integrative model, the vomeronasal pathway, which is involved in the control of reproductive physiology and behavior. The fact that brain sex differences take place in complex neural networks will help to develop a motivational theory of sex differences in reproductive behaviors. We also address the classic genomic actions in which three agents (the hormone, the intracellular receptor, and the transcription function) play an important role in brain differentiation, but we also point out refinements that such a theory requires if we want to account of the existence of two morphological patterns of sex differences in the brain, one in which males show greater morphological measures (neuron numbers and/or volume) than females and the opposite. Moreover, we also consider very important processes closely related to neuronal afferent input and membrane excitability for the developing of sex differences. Neurotransmission associated to metabotropic and ionotropic receptors, neurotrophic factors, neuroactive steroids that alter membrane excitability, cross-talk (and/or by-pass) phenomena, and second messenger pathways appear to be involved in the development of brain sex differences. The sexual differentiation of the brain and reproductive behavior is regarded as a cellular multisignaling process."
- Cognitive ability and cerebral lateralisation in transsexuals
    - "It is still unclear to what extent cross-gender identity is due to pre- and perinatal organising effects of sex hormones on the brain. Empirical evidence for a relationship between prenatal hormonal influences and certain aspects of gender typical (cognitive) functioning comes from pre- and postpubertal clinical samples, such as women suffering from congenital adrenal hyperplasia and studies in normal children. In order to further investigate the hypothesis that cross-gender identity is influenced by prenatal exposure to (atypical) sex steroid levels we conducted a study with early onset, adult male-to-female and female-to-male transsexuals, who were not yet hormonally treated,

and nontranssexual adult female and male controls. The aim of the study was to find out whether early onset transsexuals performed in congruence with their biological sex or their gender identity. The results on different tests show that gender differences were pronounced, and that the two transsexual groups occupied a position in between these two groups, thus showing a pattern of performance away from their biological sex. The findings provide evidence that organisational hormonal influences may have an effect on the development of cross-gender identity."

- Human behavioral sex differences: a role for gonadal hormones during early development?
    - "Evidence that gonadal hormones during prenatal and neonatal development influence behavior is reviewed. Several theoretical models of hormonal influences, derived from research in other species, are described. These models are evaluated on the basis of data from humans with either normal or abnormal hormonal exposure. It is concluded that the evidence is insufficient to determine which model best explains the data. Sexual differentiation may involve several dimensions, and different models may apply to different behaviors. Gonadal hormones appear to influence development of some human behaviors that show sex differences. The evidence is strongest for childhood play behavior and is relatively strong for sexual orientation and tendencies toward aggression. Also, high levels of hormones do not enhance intelligence, although a minimum level may be needed for optimal development of some cognitive processes. Directions for future research are proposed."
- Roles of steroid hormones and their receptors in structural organization in the nervous system
    - "Due to their chemical properties, steroid hormones cross the blood-brain barrier where they have profound effects on neuronal development and reorganization both in invertebrates and vertebrates, including humans mediated through their receptors. Steroids play a crucial role in the organizational actions of cellular differentiation representing sexual dimorphism and apoptosis, and in the activational effects of phenotypic changes in association with structural plasticity. Their sites of action are primarily the genes themselves but some are coupled with membrane-bound receptor/ion channels. The effects of steroid hormones on gene transcription are not direct, and other cellular components interfere with their receptors through cross-talk and convergence of the signaling pathways in neurons. These genomic and non-genomic actions account for the divergent effects of steroid hormones on brain function as well as on their structure. This review looks again at and updates the tremendous advances made in recent decades on the study of the role of steroid (gonadal and adrenal) hormones and their receptors on developmental processes and plastic changes in the nervous system."
- Early Androgens Are Related to Childhood Sex-Typed Toy Preferences - Sheri A. Berenbaum, Melissa Hines, 1992
    - "Girls with congenital adrenal hyperplasia (CAH) who were exposed to high levels of androgen in the prenatal and early postnatal periods showed increased play with boys" toys and reduced play with girls' toys compared with their unexposed female relatives at ages 3 to 8. Boys with CAH did not differ from their male relatives in play with boys' or girls' toys. These results suggest that early hormone exposure in females has a masculinizing effect on sex-typed toy preferences."

- Gene- and environment-dependent neuroendocrine etiogenesis of homosexuality and transsexualism
    - "Sexual brain organization is dependent on sex hormone and neurotransmitter levels occurring during critical developmental periods. The higher the androgen levels during brain organization, caused by genetic and/or environmental factors, the higher is the biological predisposition to bi- and homosexuality or even transsexualism in females and the lower it is in males. Adrenal androgen excess, leading to heterotypical sexual orientation and/or gender role behavior in genetic females, can be caused by 21-hydroxylase deficiency, especially when associated with prenatal stress. The cortisol (F) precursor 21-deoxycortisol (21-DOF) was found to be significantly increased after ACTH stimulation in homosexual as compared to heterosexual females. 21-DOF was increased significantly before and even highly significantly after ACTH stimulation in female-to-male transsexuals. In view of these data, heterozygous and homozygous forms, respectively, of 21-hydroxylase deficiency represent a genetic predisposition to androgen-dependent development of homosexuality and transsexualism in females. Testicular androgen deficiency in prenatal life, giving rise to heterotypical sexual orientation and/or gender role behavior in genetic males, may be induced by prenatal stress and/or maternal or fetal genetic alterations. Most recently, in mothers of homosexual men--following ACTH stimulation--a significantly increased prevalence of high 21-DOF plasma values and 21-DOF/F ratios was found, which surpassed the mean + 1 SD level of heterosexual control women. In homosexual men as well--following ACTH stimulation--most of the 21-DOF plasma values and 21-DOF/F ratios also surpassed the mean + 1 SD level of heterosexual men. In only one out of 9 homosexual males, neither in his blood nor in that of his mother increased 21-DOF values and 21-DOF/F ratios were found after ACTH stimulation. In this homosexual man, however, the plasma dehydroepiandrosterone sulfate (DHEA-S) values and the DHEA-S/1000 x A (A = androstenedione) ratio were increased before and after ACTH stimulation. Furthermore, highly significantly increased basal plasma levels of dehydroepiandrosterone sulfate were found in male-to-female transsexuals as compared to normal males, suggesting partial 3 beta-ol hydroxysteroid dehydrogenase deficiency to be a predisposing factor for the development of male-to-female transsexualism."
- Prenatal hormones versus postnatal socialization by parents as determinants of male-typical toy play in girls with congenital adrenal hyperplasia
    - "Toy choices of 3- to 10-year-old children with congenital adrenal hyperplasia (CAH) and of their unaffected siblings were assessed. Also assessed was parental encouragement of sex-typed toy play. Girls with CAH displayed more male-typical toy choices than did their unaffected sisters, whereas boys with and without CAH did not differ. Mothers and fathers encouraged sex-typical toy play in children with and without CAH. However, girls with CAH received more positive feedback for play with girls' toys than did unaffected girls. Data show that increased male-typical toy play by girls with CAH cannot be explained by parental encouragement of male-typical toy play. Although parents encourage sex-appropriate behavior, their encouragement appears to be insufficient to override the interest of girls with CAH in cross-sexed toys."
- Gender change in 46,XY persons with 5alpha-reductase-2 deficiency and 17beta-hydroxysteroid dehydrogenase-3 deficiency.

    - "Individuals with 5alpha-reductase-2 deficiency (5alpha-RD-2) and 17beta-hydroxysteroid dehydrogenase-3 deficiency (17beta-HSD-3) are often raised as girls. Over the past number of years, this policy has been challenged because many individuals with these conditions develop a male gender identity and make a gender role change after puberty. The findings also raised doubts regarding the hypothesis that children are psychosexually neutral at birth and emphasized the potential role of prenatal brain exposure to androgens in gender development. If prenatal exposure to androgens is a major contributor to gender identity development, one would expect that all, or nearly all, affected individuals, even when raised as girls, would develop a male gender identity and make a gender role switch later in life. However, an estimation of the prevalence of gender role changes, based on the current literature, shows that gender role changes occur frequently, but not invariably. Gender role changes were reported in 56-63% of cases with 5alpha-RD-2 and 39-64% of cases with 17beta-HSD-3 who were raised as girls. The changes were usually made in adolescence and early adulthood. In these two syndromes, the degree of external genital masculinization at birth does not seem to be related to gender role changes in a systematic way."
- Prenatal phthalate exposure and reduced masculine play in boys
    - "Foetal exposure to antiandrogens alters androgen-sensitive development in male rodents, resulting in less male-typical behaviour. Foetal phthalate exposure is also associated with male reproductive development in humans, but neurodevelopmental outcomes have seldom been examined in relation to phthalate exposure. To assess play behaviour in relation to phthalate metabolite concentration in prenatal urine samples, we recontacted participants in the Study for Future Families whose phthalate metabolites had been measured in mid-pregnancy urine samples. Mothers completed a questionnaire including the Pre-School Activities Inventory, a validated instrument used to assess sexually dimorphic play behaviour. We examined play behaviour scores (masculine, feminine and composite) in relationship to (log10) phthalate metabolite concentrations in mother's urine separately for boys ($N = 74$) and girls ($N = 71$). Covariates (child's age, mother's age and education and parental attitude towards atypical play choices) were controlled using multivariate regression models. Concentrations of dibutyl phthalate metabolites, mono-*n*-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP) and their sum, were associated with a decreased (less masculine) composite score in boys (regression coefficients −4.53,−3.61 and −4.20, $p = 0.01$, 0.07 and 0.04 for MnBP, MiBP and their sum respectively). Concentrations of two urinary metabolites of di(2-ethylhexyl) phthalate (DEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and the sum of these DEHP metabolites plus mono(2-ethylhexyl) phthalate were associated with a decreased masculine score (regression coefficients −3.29,−2.94 and −3.18, $p = 0.02$, 0.04 and 0.04) for MEHHP, MEOHP and the sum respectively. No strong associations were seen between behaviour and urinary concentrations of any other phthalate metabolites in boys, or between girls' scores and any metabolites. These data, although based on a small sample, suggest that prenatal exposure to antiandrogenic phthalates may be associated with less male-typical play behaviour in boys. Our findings suggest that these ubiquitous environmental chemicals have the potential to alter androgen-responsive brain development in humans."
- Sex steroid-related genes and male-to-female transsexualism

    - "Transsexualism is characterised by lifelong discomfort with the assigned sex and a strong identification with the opposite sex. The cause of transsexualism is unknown, but it has been suggested that an aberration in the early sexual differentiation of various brain structures may be involved. Animal experiments have revealed that the sexual differentiation of the brain is mainly due to an influence of testosterone, acting both via androgen receptors (ARs) and—after aromatase-catalyzed conversion to estradiol—via estrogen receptors (ERs). The present study examined the possible importance of three polymorphisms and their pairwise interactions for the development of male-to-female transsexualism: a CAG repeat sequence in the first exon of the AR gene, a tetra nucleotide repeat polymorphism in intron 4 of the aromatase gene, and a CA repeat polymorphism in intron 5 of the ERβ gene. Subjects were 29 Caucasian male-to-female transsexuals and 229 healthy male controls. Transsexuals differed from controls with respect to the mean length of the ERβ repeat polymorphism, but not with respect to the length of the other two studied polymorphisms. However, binary logistic regression analysis revealed significant partial effects for all three polymorphisms, as well as for the interaction between the AR and aromatase gene polymorphisms, on the risk of developing transsexualism. Given the small number of transsexuals in the study, the results should be interpreted with the utmost caution. Further study of the putative role of these and other sex steroid-related genes for the development of transsexualism may, however, be worthwhile."
- Behavioral Sexual Dimorphism in School-Age Children and Early Developmental Exposure to Dioxins and PCBs: A Follow-Up Study of the Duisburg Cohort
    - "*Results:* Mean blood levels of summed WHO2005-TEQ–standardized dioxins (ΣPCDD/Fs) were 14.5 ± 6.4 pg/g blood lipids, and ΣPCBs were 6.9 ± 3.8 pg/g blood lipids, with similar values for milk lipids. Regression analyses revealed some highly significant interactions between sex and exposure—such as for ΣPCBs in milk, pronounced positive (boys: β = 3.24; CI = 1.35, 5.14) or negative (girls: β = −3.59; CI = −1.10, −6.08) associations with reported femininity. Less pronounced and mostly insignificant but consistent associations were found for the masculinity score, positive for boys and negative for girls.
      Conclusions: Given our results and the findings of previous studies, we conclude that there is sufficient evidence that these EDCs modify behavioral sexual dimorphism in children, presumably by interacting with the hypothalamic–pituitary–gonadal axis."
- Sex specific effect of prenatal testosterone on language lateralization in children
    - "Brain lateralization refers to the division of labour between the two hemispheres in controlling a wide array of functions and is remarkably well developed in humans. Based on sex differences in lateralization of handedness and language, several hypotheses have postulated an effect of prenatal exposure to testosterone on human lateralization development, the topic of a long-standing and unresolved debate. Here we demonstrate a clear relationship between prenatal levels of testosterone as assessed from amniotic fluid of healthy pregnant mothers and language lateralization of their offspring at the age of 6 years. Using focused attention conditions in the dichotic listening task, in which the child is instructed to report information from the left ear or the right ear, we were able to differentiate between potential effects of early testosterone on the left hemisphere and effects on inter-hemispheric connectivity. This provides a new method to distinguish between the claims of the different hypotheses. The results suggest that in girls higher

prenatal testosterone exposure facilitates left hemisphere language processing, whereas in boys it reduces the information transfer via the corpus callosum."

- Differential effects of prenatal testosterone on lateralization of handedness and language
    - "*Results:* Results demonstrate that higher pT exposure was related to a decrease in strength of handedness (R^2 = .11, p = .01). The analysis shows that pT has quite stronger explanatory power than sex by itself, although there may be an additional effect of sex independent from pT. In a subgroup of these children we recently reported that higher levels of pT are related to increased left hemisphere dominance for language. Analyses show that pT is differentially related to handedness and language lateralization in these children (Z > 2.75, p < .003).
      Conclusions: Results imply a differential effect of pT on language lateralization and handedness. This may be explained by differential sensitivity of different areas of the corpus callosum or hemispheres for androgens, fuelling the ongoing debate about the relationship between prenatal exposure to testosterone and lateralization of brain and behavior."
- Sex differences in children's free drawings: a study on girls with congenital adrenal hyperplasia
    - "Sex differences are recognized in children's free drawings with respect to motifs, colors, figure compositions, and expression. Boys tend to draw mobile objects and mechanical objects with dark or cold colors and often use bird's-eye-view composition when they draw pictures, whereas girls like to draw human motifs (especially girls and women), flowers, and butterflies with light and warm colors and tend to arrange motifs in a row on the ground. Analyses were made on the drawings of girls with congenital adrenal hyperplasia (CAH) and unaffected boys and girls, using masculine and feminine indexes. Sex difference in masculine and feminine indexes was clear in the drawings by unaffected boys and girls. Their drawings do not or mostly do not contain characteristics typical of the opposite sex. Compared with those of unaffected girls, the pictures of CAH girls more strongly showed masculine characteristics. The feminine index for the pictures of CAH girls was significantly lower than that for unaffected girls, while the masculine index for CAH girls was significantly higher than that for unaffected girls. Furthermore, the masculine index for CAH girls was not significantly different from that of unaffected boys. These results suggest that androgen exposure during fetal life may contribute to shaping masculine characteristics in children's free drawings."
- Gender identity in XY intersexuality
    - "The following syndromes of XY intersexuality are reviewed: 5alpha-reductase-2 deficiency, 17beta-hydroxysteroid dehydrogenase-3 deficiency, and complete and partial androgen insensitivity with attention focused on issues of gender identity. Each syndrome, with its unique presentation, provides an opportunity to explore the relative effects of nature (androgens) versus nurture (sex of rearing) in gender identity development. The phenomenon of gender role reversal in these conditions is described and theories on the determinants of gender identity formation are proposed. Issues of importance to psychiatrists in treating patients who have these conditions also are discussed."
- A study of gender outcome of Egyptian patients with 46,XY disorder of sex development

    - "Children with disorder of sex development (DSD) may be born with ambiguous genitalia. Decision-making in relation to sex assignment has been perceived as extremely disturbing and difficult to families and health care professionals. This is mainly due to a general paucity of information about the condition and an exaggerated feeling of stigma and shame associated with genital abnormalities. This is the first study in Egypt aimed at studying the psychosexual development and gender outcome of 40 Egyptian patients with 46,XY DSD focusing on the impact of social and religious factors. The patients were subjected to history-taking, pedigree analysis, full clinical examination, and cytogenetic studies. Hormonal, radiological investigations and molecular studies were performed when possible. Accordingly, they were classified into 4 groups: (1) sex chromosome aneuploid DSD (mixed gonadal dysgenesis) and (2) disorders of gonadal development (gonadal dysgenesis); (3) androgen biosynthesis defect (5alpha-reductase deficiency, 17beta-hydroxysteroid dehydrogenase deficiency), and (4) defect in androgen action (androgen insensitivity syndrome). The psychosexual development was assessed using adapted structured questionnaire and the Bem sex role inventory for patients below and above 12 years of age, respectively. Thirty-two patients (80%) were initially assigned as females; 3 patients with gonadal dysgenesis, 1 patient with 5alpha-reductase deficiency, and 1 patient with androgen insensitivity were reassigned as male. Male reassignment also was recorded in 5 patients with 17beta-hydroxysteroid dehydrogenase deficiency and one of them showed sex reversal twice. Gender outcome of our patients is elusive; the social component has a significant impact on the gender outcome in our society, even more than religion. We recommend that in the future more and more patients should be analyzed as well. These studies should be designed to emphasize the quality of life of DSD patients."
- Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5alpha-reductase deficiency
    - "To determine the contribution of androgens to the formation of male-gender identity, we studied male pseudohermaphrodites who had decreased dihydrotestosterone production due to 5 alpha-reductase deficiency. These subjects were born with female-appearing external genitalia and were raised as girls. They have plasma testosterone levels in the high normal range, show an excellent response to testosterone and are unique models for evaluating the effect of testosterone, as compared with a female upbringing, in determining gender identity. Eighteen of 38 affected subjects were unambiguously raised as girls, yet during or after puberty, 17 of 18 changed to a male-gender identity and 16 of 18 to a male-gender role. Thus, exposure of the brain to normal levels of testosterone in utero, neonatally and at puberty appears to contribute substantially to the formation of male-gender identity. These subjects demonstrate that in the absence of sociocultural factors that could interrupt the natural sequence of events, the effect of testosterone predominates, over-riding the effect of rearing as girls."
- True hermaphroditism: from female to male endocrine status
    - "True hermaphroditism was revealed by monthly intrascrotal bleeding in a 21-yr-old subject of male phenotype who had undergone surgical treatment for gonadal ectopy at the age of 7 yr. The presence of an ovary was demonstrated by the endocrine profile of an ovulatory menstrual cycle. Evidence for the presence of a testis was provided by a plasma testosterone increase after hCG administration (5000 IU/day for 3 days) and its spontaneous response to an endogenous preovulatory LH peak. Further endocrine

studies revealed that both gonads were stimulated by endogenous gonadotropins. At surgery, a hemiuterus and an ovary with corpus luteum were found in the left hemiscrotum, and a testis and epididymis were found in the right hemiscrotum. After removal of the ovary, the subject passed from a predominantly female to a male endocrine status, which suggests that the endocrine secretion of the testis was inhibited by the negative feedback effect of ovarian steroids on gonadotropin secretion."

- [True hermaphroditism]
    - "True hermaphroditism in humans is defined as the simultaneous presence of both testicular and ovarian tissue in a single individual. I reviewed clinical findings, karyotype, findings of recent molecular analysis, diagnostic tools and treatment of true hermaphroditism. Recent molecular analysis in true hermaphroditism revealed that duplication of 22q was recognized in a 46,XX SRY-negative case. A 46,XX true hermaphrodite in which SRY was negative in blood leukocytes and epithelial oral cells but present and partially deleted in DNA obtained from the testicular portion of the ovotestes was reported. And also, the study using PCR and FISH analyses revealed the presence of hidden mosaicism for SRY or other Y sequences in some patients with XX true hermaphroditism and mosaicism for SRY limited to the gonads is an alternative mechanism for testicular development in 46,XX true hermaphrodites."
- True hermaphroditism: geographical distribution, clinical findings, chromosomes and gonadal histology
    - "We reviewed 283 cases of human true hermaphroditism published from 1980 to 1992. Of the 96 cases described in Africa 96.9% showed a 46,XX karyotype. In Europe 40.5% of 74 cases and 21.0% of the patients in North America had chromosomal mosaicism. The 46,XY karyotype is extremely rare (7%) and equally distributed through Asia, Europe and North America. Of 283 cases 87 were of black or black mixed origin with a 46,XX chromosomal constellation. The most common gonad in patients with true hermaphroditism, an ovotestis, was found in 44.4% of 568 gonads. Gonads with testicular tissue were more frequent on the right side of the body, while pure ovarian tissue was more common on the left. Histologically the testicular tissue was described to be immature and only twice was spermatogenesis reported while the ovarian portion often appeared normal. This coincides with 21 pregnancies reported in ten true hermaphrodites while only one true hermaphrodite apparently has fathered a child. Of the patients 4.6% were reported to have gonadal tumours. Position and type of the genital ducts, frequency of clinical findings such as genital abnormalities and gynaecomastia, correlations between assigned sex and karyotype as well as the age at diagnosis are reported."
- It is not all hormones: alternative explanations for sexual differentiation of the brain
    - "Males and females of many species differ with regard to neurodevelopment, ongoing brain function and behavior. For many years, it was assumed that these differences primarily arose due to hormonal masculinization of the male brain (and to a lesser extent hormonal feminization of the female brain). Recent elegant experiments in model systems have revealed that, while gonadal hormones undoubtedly play an important role in sexual differentiation of the brain, they are not the only possible mechanism for this phenomenon. In the present review, we discuss the concept that genes residing upon the sex chromosomes (which are asymmetrically inherited between males and females) may influence sexually dimorphic neurobiology directly, and suggest possible mechanisms.

Future work will be directed towards understanding the extent and specificity with which sex-linked genes and hormones define brain structure and function, and towards elucidating potential interactions between the two mechanisms. Ultimately, it is hoped that such studies will provide insights into why men and women are differentially vulnerable to certain mental disorders, and will enable the development of effective sex-tailored therapeutics."

- From gene networks underlying sex determination and gonadal differentiation to the development of neural networks regulating sociosexual behavior
    - "Genes are not expressed in isolation any more than social behavior has meaning outside of society. Both are in dynamic flux with the immediate environment that the gene/individual finds itself, which in turn establishes the timing, pattern, and conditions of expression. This means that complex behaviors and their genetic underpinnings should be viewed as a cumulative process, or as the result of experiences up to that point in time and, at the same time, as setting the stage for what will follow. The evidence indicates that as experiences accumulate throughout life, early experiences shape how genes/individuals will respond to later experiences, whereas later experiences modify the effects of these earlier experiences. A method of graphically representing and analyzing change in gene and neural networks is presented. Results from several animal model systems will be described to illustrate these methods. First, we will consider the phenomenon of temperature-dependent sex determination in reptiles. We will illustrate how the experience of a particular temperature during a sensitive period of embryogenesis sculpts not only the patterns of expression of genes involved in sex determination and gonadal differentiation but also the morphological, physiological, neuroendocrine, and behavioral traits of the adult phenotype. The second model system concerns the effects of the sex ratio in the litter in rats, and the genotype ratio in the litter of transgenic mice, on the nature and frequency of maternal care and how this in turn influences the patterns of activation of identified neural circuits subserving the offspring's sociosexual behavior when it is an adult."
- Endocrine-disrupting chemicals: Effects on neuroendocrine systems and the neurobiology of social behavior
    - "Endocrine-disrupting chemicals (EDCs) are pervasive in the environment. They are found in plastics and plasticizers (bisphenol A (BPA) and phthalates), in industrial chemicals such as polychlorinated biphenyls (PCBs), and include some pesticides and fungicides such as vinclozolin. These chemicals act on hormone receptors and their downstream signaling pathways, and can interfere with hormone synthesis, metabolism, and actions. Because the developing brain is particularly sensitive to endogenous hormones, disruptions by EDCs can change neural circuits that form during periods of brain organization. Here, we review the evidence that EDCs affect developing hypothalamic neuroendocrine systems, and change behavioral outcomes in juvenile, adolescent, and adult life in exposed individuals, and even in their descendants. Our focus is on social, communicative and sociosexual behaviors, as how an individual behaves with a same- or opposite-sex conspecific determines that individual's ability to exist in a community, be selected as a mate, and reproduce successfully."
- Effects of perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age

    - "Polychlorinated biphenyls (PCBs) and dioxins are known as neurotoxic compounds that may modulate sex steroid hormones. Steroid hormones play a mediating role in brain development and may influence behaviors that show sex differences, such as childhood play behavior. In this study we evaluated the effects of perinatal exposure to environmental levels of PCBs and dioxins on childhood play behavior and whether the effects showed sex differences. As part of the follow-up to the Dutch PCB/dioxin study at school age, we used the Pre-School Activity Inventory (PSAI) to assess play behavior in the Rotterdam cohort (n = 207). The PSAI assesses masculine or feminine play behavior scored on three subscales: masculine, feminine, and composite. Prenatal exposure to PCBs was defined as the sum of PCB 118, 138, 153, and 180 in maternal and cord plasma and breast milk. For breast milk we measured additional PCBs as well as 17 dioxins. Respondents returned 160 questionnaires (age 7.5 years +/- 0.4). Effects of prenatal exposure to PCBs, measured in maternal and cord plasma, on the masculine and composite scales were different for boys and girls ($p < .05$). In boys, higher prenatal PCB levels were related with less masculinized play, assessed by the masculine scale ($p(\text{maternal}) = .042$; $p(\text{cord}) = .001$) and composite scale ($p(\text{cord}) = .011$), whereas in girls higher PCB levels were associated with more masculinized play, assessed by the composite scale ($p(\text{PCBmilk}) = .028$). Higher prenatal dioxin levels were associated with more feminized play in boys as well as girls, assessed by the feminine scale ($p = .048$). These effects suggest prenatal steroid hormone imbalances caused by prenatal exposure to environmental levels of PCBs, dioxins, and other related organochlorine compounds."
- Review Sex steroids and human behavior: prenatal androgen exposure and sex-typical play behavior in children
    - "Gonadal hormones, particularly androgens, direct certain aspects of brain development and exert permanent influences on sex-typical behavior in nonhuman mammals. Androgens also influence human behavioral development, with the most convincing evidence coming from studies of sex-typical play. Girls exposed to unusually high levels of androgens prenatally, because they have the genetic disorder, congenital adrenal hyperplasia (CAH), show increased preferences for toys and activities usually preferred by boys, and for male playmates, and decreased preferences for toys and activities usually preferred by girls. Normal variability in androgen prenatally also has been related to subsequent sex-typed play behavior in girls, and nonhuman primates have been observed to show sex-typed preferences for human toys. These findings suggest that androgen during early development influences childhood play behavior in humans at least in part by altering brain development."
- Effects of sex on object recognition and spatial navigation in humans
    - "Human tests designed to mirror rodent tests of object recognition and spatial navigation were administered to adult cognitively healthy humans. Facial recognition was also assessed. There was no sex difference in facial recognition, consistent with earlier studies. In the object recognition test, the test-retest NINL total scores during the same visit were highly correlated, comparable to the test-retest correlations obtained in the established facial recognition test. There were no effects of sex on object recognition. However, in the spatial navigation test, there were effects of sex on spatial learning and memory during the session with the hidden, but not visible, target. These tests might be

useful to compare assessments of object recognition and spatial learning and memory in humans and animal models."

- Comparing Postnatal Development of Gonadal Hormones and Associated Social Behaviors in Rats, Mice, and Humans
    - "Postnatal development includes dramatic changes in gonadal hormones and the many social behaviors they help regulate, both in rodents and humans. Parental care-seeking is the most salient social interaction in neonates and infants, play and prosocial behaviors are commonly studied in juveniles, and the development of aggression and sexual behavior begins in peripubertal stages but continues through late adolescence into adulthood. Although parental behaviors are shown after reproductive success in adulthood, alloparenting behaviors are actually high in juveniles as well. These behaviors are sensitive to both early-life organizational effects of gonadal hormones and later-life activational regulation. However, changes in circulating gonadal hormones and the display of the previous behaviors over development differ between rats, mice, and humans. These endpoints are of interest to endocrinologist, toxicologists, and neuroscientists because of their relevance to mental health disorders and their vulnerability to effects of endocrine-disrupting chemical exposure. As such, the goal of this mini-review is to succinctly describe and relate the postnatal development of gonadal hormones and social behaviors to each other, over time, and across animal models. Ideally, this will help identify appropriate animal models and age ranges for continued study of both normative development and in contexts of environmental disruption."
- Psychological outcomes and gender-related development in complete androgen insensitivity syndrome
    - "We evaluated psychological outcomes and gender development in 22 women with complete androgen insensitivity syndrome (CAIS). Participants were recruited through a medical database (n = 10) or through a patient support group (n = 12). Controls included 14 males and 33 females, of whom 22 were matched to women with CAIS for age, race, and sex-of-rearing. Outcome measures included quality of life (self-esteem and psychological general well-being), gender-related psychological characteristics (gender identity, sexual orientation, and gender role behavior in childhood and adulthood), marital status, personality traits that show sex differences, and hand preferences. Women recruited through the database versus the support group did not differ systematically, and there were no statistically significant differences between the 22 women with CAIS and the matched controls for any psychological outcome. These findings argue against the need for two X chromosomes or ovaries to determine feminine-typical psychological development in humans and reinforce the important role of the androgen receptor in influencing masculine-typical psychological development. They also suggest that psychological outcomes in women with CAIS are similar to those in other women. However, additional attention to more detailed aspects of psychological well-being in CAIS is needed."
- An enlarged suprachiasmatic nucleus in homosexual men
    - "Morphometric analysis of the human hypothalamus revealed that the volume of the suprachiasmatic nucleus (SCN) in homosexual men is 1.7 times as large as that of a reference group of male subjects and contains 2.1 times as many cells. In another hypothalamic nucleus which is located in the immediate vicinity of the SCN, the sexually

dimorphic nucleus (SDN), no such differences in either volume or cell number were found. The SDN data indicate the selectivity of the enlarged SCN in homosexual men, but do not support the hypothesis that homosexual men have a 'female hypothalamus'."

Animal Studies

- Gene Expression Profile of the Neonatal Female Mouse Brain After Administration of Testosterone Propionate
    - "Differences in genes that are expressed differentially following administration of testosterone injection from known sexually dimorphic genes suggest that many GD-related genes are upregulated during female brain masculinization. The gene set identified in this study provides a basis to better understand the mechanisms of GD and delineate its associated biomarkers. "
- Perinatal Administration of Aromatase Inhibitors in Rodents as Animal Models of Human Male Homosexuality: Similarities and Differences
    - "Recently, we have established that the treatment with low doses of letrozole during the second half of pregnancy produces male rat offspring, that when adults spend more time in the company of a sexually active male than with a receptive female in a preference test. In addition, they display female sexual behavior when forced to interact with a sexually experienced male and some typical male sexual behavior when faced with a sexually receptive female. Interestingly, these males displayed both sexual behavior patterns spontaneously, i.e., in absence of exogenous steroid hormone treatment. Most of these features correspond with those found in human male homosexuals; however, the "bisexual" behavior shown by the letrozole-treated rats may be related to a particular human population. All these data, taken together, permit to propose letrozole prenatal treatment as a suitable animal model to study human male homosexuality and reinforce the hypothesis that human sexual orientation is underlined by changes in the endocrine milieu during early development."
- Sexual Differentiation of the Brain Requires Perinatal Kisspeptin-GnRH Neuron Signaling
    - "Sex differences in brain function underlie robust differences between males and females in both normal and disease states. Although alternative mechanisms exist, sexual differentiation of the male mammalian brain is initiated predominantly by testosterone secreted by the testes during the perinatal period. Despite considerable advances in understanding how testosterone and its metabolite estradiol sexually differentiate the brain, little is known about the mechanism that generates the male-specific perinatal testosterone surge. In mice, we show that a male-specific activation of GnRH neurons occurs 0–2 h following birth and that this correlates with the male-specific surge of testosterone occurring up to 5 h after birth. The necessity of GnRH signaling for the sexually differentiating effects of the perinatal testosterone surge was demonstrated by the persistence of female-like brain characteristics in adult male, GnRH receptor knock-out mice. Kisspeptin neurons have recently been identified to be potent, direct activators of GnRH neurons. We demonstrate that a population of kisspeptin neurons appears in the preoptic area of only the male between E19 and P1. The importance of kisspeptin inputs to GnRH neurons for the process of sexual differentiation was demonstrated by the lack of a normal neonatal testosterone surge, and disordered brain sexual differentiation of male mice in which the kisspeptin receptor was deleted

selectively from GnRH neurons. These observations demonstrate the necessity of perinatal GnRH signaling for driving brain sexual differentiation and indicate that kisspeptin inputs to GnRH neurons are essential for this process to occur."

- The mechanisms underlying sexual differentiation of behavior and physiology in mammals and birds: relative contributions of sex steroids and sex chromosomes
    - "From a classical viewpoint, sex-specific behavior and physiological functions as well as the brain structures of mammals such as rats and mice, have been thought to be influenced by perinatal sex steroids secreted by the gonads. Sex steroids have also been thought to affect the differentiation of the sex-typical behavior of a few members of the avian order Galliformes, including the Japanese quail and chickens, during their development in ovo. However, recent mammalian studies that focused on the artificial shuffling or knockout of the sex-determining gene, Sry, have revealed that sex chromosomal effects may be associated with particular types of sex-linked differences such as aggression levels, social interaction, and autoimmune diseases, independently of sex steroid-mediated effects. In addition, studies on naturally occurring, rare phenomena such as gynandromorphic birds and experimentally constructed chimeras in which the composition of sex chromosomes in the brain differs from that in the other parts of the body, indicated that sex chromosomes play certain direct roles in the sex-specific differentiation of the gonads and the brain. In this article, we review the relative contributions of sex steroids and sex chromosomes in the determination of brain functions related to sexual behavior and reproductive physiology in mammals and birds."
- Testosterone exposure during the critical period decreases corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of female rats
    - "We previously described sex differences in the number of corticotropin-releasing hormone-immunoreactive (CRH-ir) neurons in the dorsolateral division of the bed nucleus of the stria terminalis (BSTLD). Female rats were found to have more CRH neurons than male rats. We hypothesized that testosterone exposure during the critical period of sexual differentiation of the brain decreased the number of CRH-ir neurons in the hypothalamus, including the BSTLD and preoptic area. In the present study we confirm that testosterone exposure during the neonatal period results in changes to a variety of typical aspects of the female reproductive system, including estrous cyclicity as shown by virginal smear, the positive feedback effects of estrogen alone or combined with progesterone, luteinizing hormone secretions, and estrogen and progesterone-induced Fos expression in gonadotropin-releasing hormone neurons. The number of CRH-ir neurons in the preoptic area did not change, whereas CRH-ir neurons in the BSTLD significantly decreased in estrogen-primed ovariectomized rats exposed to testosterone during the neonatal period. These results suggest that the sexual differentiation of CRH neurons in the BSTLD is a result of testosterone exposure during the critical period and the BSTLD is more fragile than the preoptic area during sexual differentiation. Furthermore, sex differences in CRH in the preoptic area may not be caused by testosterone during this period."
- Effects of prenatal androgens on rhesus monkeys: a model system to explore the organizational hypothesis in primates. - Abstract
    - "After proposing the organizational hypothesis from research in prenatally androgenized guinea pigs (Phoenix, C.H., Goy, R.W., Gerall, A.A., Young, W.C., 1959. Organizational

action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65, 369-382.), the same authors almost immediately extended the hypothesis to a nonhuman primate model, the rhesus monkey. Studies over the last 50 years have verified that prenatal androgens have permanent effects in rhesus monkeys on the neural circuits that underlie sexually dimorphic behaviors. These behaviors include both sexual and social behaviors, all of which are also influenced by social experience. Many juvenile behaviors such as play, mounting, and vocal behaviors are masculinized and/or defeminized, and aspects of adult sexual behavior are both masculinized (e.g. approaches, sex contacts, and mounts) and defeminized (e.g. sexual solicits). Different behavioral endpoints have different periods of maximal susceptibility to the organizing actions of prenatal androgens. Aromatization is not important, as both testosterone and dihydrotestosterone are equally effective in rhesus monkeys. Although the full story of the effects of prenatal androgens on sexual and social behaviors in the rhesus monkey has not yet completely unfolded, much progress has been made. Amazingly, a large number of the inferences drawn from the original 1959 study have proved applicable to this nonhuman primate model."

- Juvenile Rank Can Predict Male-Typical Adult Mating Behavior in Female Sheep Treated Prenatally with Testosterone
    - "Previous research with female sheep indicates that exposure to excess testosterone for 60 days (from Gestational Days 30-90 of the 147-day gestation) leads to virilized genitalia, severe neuroendocrine deficits, as well as masculinization and defeminization of sexual behavior (T60 females). In contrast, 30 days of testosterone exposure (Gestational Days 60-90) produce animals with female-typical genitalia, less severe neuroendocrine alterations, and variable gender patterns of sexual behavior (T30 females). Variation in adult sexual behavior of male ungulates is influenced by early social experience, but this has never been tested in females. Here we investigate the influence of rank in the dominance hierarchy on the expression of adult sexual behavior in females. Specifically, we hypothesized that juvenile rank would predict the amount of male- and female-typical mating behavior exhibited by adult female sheep. This hypothesis was tested in two treatment groups and their controls (group 1: T60 females; group 2: T30 females). Dominance hierarchies were determined by observing competition over resources. Both groups of prenatal testosterone-treated females were higher ranking than controls (T60: $P = 0.05$; T30: $P < 0.01$). During the breeding season, both T60 and T30 females exhibited more male-typical mating behavior than did controls; however, the T30 animals also exhibited female-typical behavior. For the T60 group, prenatal treatment, not juvenile rank, best predicted male-typical sex behavior ($P = 0.007$), while juvenile rank better predicted male mating behavior for the T30 group ($P = 0.006$). Rank did not predict female mating behavior in the hormone-treated or control ewes. We conclude that the effect of prenatal testosterone exposure on adult male-specific but not female-specific mating behavior is modulated by juvenile social experiences."
- Sexual Differentiation of Behaviour in Monkeys: Role of Prenatal Hormones
    - "The theoretical debate over the relative contributions of nature and nurture to the sexual differentiation of behaviour has increasingly moved towards an interactionist explanation that requires both influences. In practice, however, nature and nurture have often been seen as separable, influencing human clinical sex assignment decisions, sometimes with disastrous consequences. Decisions about the sex assignment of children born with

intersex conditions have been based almost exclusively on the appearance of the genitals and how other's reactions to the gender role of the assigned sex affect individual gender socialisation. Effects of the social environment and gender expectations in human cultures are ubiquitous, overshadowing the potential underlying biological contributions in favour of the more observable social influences. Recent work in nonhuman primates showing behavioural sex differences paralleling human sex differences, including toy preferences, suggests that less easily observed biological factors also influence behavioural sexual differentiation in both monkeys and humans. We review research, including Robert W. Goy's pioneering work with rhesus monkeys, which manipulated prenatal hormones at different gestation times and demonstrated that genital anatomy and specific behaviours are independently sexually differentiated. Such studies demonstrate that, for a variety of behaviours, including juvenile mounting and rough play, individuals can have the genitals of one sex but show the behaviour more typical of the other sex. We describe another case, infant distress vocalisations, where maternal responsiveness is best accounted for by the mother's response to the genital appearance of her offspring. Taken together, these studies demonstrate that sexual differentiation arises from complex interactions where anatomical and behavioural biases, produced by hormonal and other biological processes, are shaped by social experience into the behavioural sex differences that distinguish males and females."

- Testosterone Programs Adult Social Behavior before and during, But Not after, Adolescence
    - "Whereas the adolescent brain is a major target for gonadal hormones, our understanding of hormonal influences on adolescent neural and behavioral development remains limited. These experiments investigated how variations in the timing of testosterone (T) exposure, relative to adolescence, alters the strength of steroid-sensitive neural circuits underlying social behavior in male Syrian hamsters. Experiment 1 simulated early, on-time, and late pubertal development by gonadectomizing males on postnatal d 10 and treating with SILASTIC brand T implants for 19 d before, during, or after adolescence. T treatment before or during, but not after, adolescence facilitated mating behavior in adulthood. In addition, preadolescent T treatments most effectively increased mating behavior overall, indicating that the timing of exposure to pubertal hormones contributes to individual differences in adult behavior. Experiment 2 examined the effects of preadolescent T treatment on behavior and brain regional volumes within the mating neural circuit of juvenile males (*i.e.* still preadolescent). Although preadolescent T treatment did not induce reproductive behavior in juvenile males, it did increase volumes of the bed nucleus of the stria terminalis, sexually dimorphic nucleus, posterodorsal medial amygdala, and posteroventral medial amygdala to adult-typical size. In contrast, juvenile anterodorsal medial amygdala and ventromedial hypothalamus volumes were not changed by preadolescent T treatment yet differed significantly in volume from adult controls, suggesting that further maturation of these brain regions during adolescence is required for the expression of male reproductive behavior. Thus, adolescent maturation of social behavior may involve both steroid-independent and -dependent processes, and adolescence marks the end of a postnatal period of sensitivity to steroid-dependent organization of the brain."
- Sex differences in rhesus monkey toy preferences parallel those of children. - Abstract

    - "Sex differences in toy preferences in children are marked, with boys expressing stronger and more rigid toy preferences than girls, whose preferences are more flexible. Socialization processes, parents, or peers encouraging play with gender-specific toys are thought to be the primary force shaping sex differences in toy preference. A contrast in view is that toy preferences reflect biologically-determined preferences for specific activities facilitated by specific toys. Sex differences in juvenile activities, such as rough-and-tumble play, peer preferences, and infant interest, share similarities in humans and monkeys. Thus if activity preferences shape toy preferences, male and female monkeys may show toy preferences similar to those seen in boys and girls. We compared the interactions of 34 rhesus monkeys, living within a 135 monkey troop, with human wheeled toys and plush toys. Male monkeys, like boys, showed consistent and strong preferences for wheeled toys, while female monkeys, like girls, showed greater variability in preferences. Thus, the magnitude of preference for wheeled over plush toys differed significantly between males and females. The similarities to human findings demonstrate that such preferences can develop without explicit gendered socialization. We offer the hypothesis that toy preferences reflect hormonally influenced behavioral and cognitive biases which are sculpted by social processes into the sex differences seen in monkeys and humans."
- The Role of Androgen Receptors in the Masculinization of Brain and Behavior: What we've learned from the Testicular Feminization Mutation
    - "Many studies demonstrate that exposure to testicular steroids such as testosterone early in life masculinizes the developing brain, leading to permanent changes in behavior. Traditionally, masculinization of the rodent brain is believed to depend on estrogen receptors (ERs) and not androgen receptors (ARs). According to the aromatization hypothesis, circulating testosterone from the testes is converted locally in the brain by aromatase to estrogens, which then activate ERs to masculinize the brain. However, an emerging body of evidence indicates that the aromatization hypothesis cannot fully account for sex differences in brain morphology and behavior, and that androgens acting on ARs also play a role. The testicular feminization mutation (Tfm) in rodents, which produces a nonfunctional AR protein, provides an excellent model to probe the role of ARs in the development of brain and behavior. Tfm rodent models indicate that ARs are normally involved in the masculinization of many sexually dimorphic brain regions and a variety of behaviors, including sexual behaviors, stress response and cognitive processing. We review the role of ARs in the development of the brain and behavior, with an emphasis on what has been learned from Tfm rodents as well as from related mutations in humans causing complete androgen insensitivity."
- Hormonal influences on sexually differentiated behavior in nonhuman primates
    - "Sexually dimorphic behavior in nonhuman primates results from behavioral predispositions organized by prenatal androgens. The rhesus monkey has been the primary primate model for understanding the hormonal organization of sexually dimorphic behavior. Historically, female fetuses have received high prenatal androgen doses to investigate the masculinizing and defeminizing effects of androgens. Such treatments masculinized juvenile and adult copulatory behavior and defeminized female-typical sexual initiation to adult estrogen treatment. Testosterone and the nonaromatizable androgen, 5alpha-dihydrotestosterone, produced similar effects suggesting that estrogenic metabolites of androgens are not critical for masculinization

and defeminization in rhesus monkeys. Long duration androgen treatments masculinized both behavior and genitalia suggesting that socializing responses to the females' male-like appearance may have produced the behavioral changes. Treatments limited to 35 days early or late in gestation differentially affected behavioral and genital masculinization demonstrating direct organizing actions of prenatal androgens. Recent studies exposed fetal females to smaller doses of androgens and interfered with endogenous androgens using the anti-androgen flutamide. Low dose androgen treatment only significantly masculinized infant vocalizations and produced no behavioral defeminization. Females receiving late gestation flutamide showed masculinized infant vocalizations and defeminized interest in infants. Both late androgen and flutamide treatment hypermasculinized some male juvenile behaviors. Early flutamide treatment blocked full male genital masculinization, but did not alter their juvenile or adult behavior. The role of neuroendocrine feedback mechanisms in the flutamide effects is discussed. Sexually differentiated behavior ultimately reflects both hormonally organized behavioral predispositions and the social experience that converts these predispositions into behavior."

- Perinatal Exposure to Low Levels of the Environmental Antiandrogen Vinclozolin Alters Sex-Differentiated Social Play and Sexual Behaviors in the Rat
    - "In this study we examined the effects of exposure to the antiandrogenic fungicide vinclozolin (Vz) on the development of two sex-differentiated behaviors that are organized by the perinatal actions of androgens. Pregnant Long-Evans rats were administered a daily oral dose of 0, 1.5, 3, 6, or 12 mg/kg Vz from the 14th day of gestation through postnatal day (PND)3. The social play behavior of juvenile offspring was examined on PND22 and again on PND34 during play sessions with a same-sex littermate. After they reached adulthood, the male offspring were examined with the *ex copula* penile reflex procedure to assess erectile function. Vz did not produce any gross maternal or neonatal toxicity, nor did it reduce the anogenital distance in male pups. We observed no effects of Vz on play behavior on PND22. However, the 12-mg/kg Vz dose significantly increased play behavior in the male offspring on PND34 compared with controls. The most dramatic increases were seen with the nape contact and pounce behavior components of play. The Vz effect was more pronounced in male than in female offspring. As adults, male offspring showed a significant reduction of erections at all dose levels during the *ex copula* penile reflex tests. The 12-mg/kg dose was also associated with an increase in seminal emissions. These effects demonstrate that perinatal Vz disrupts the development of androgen-mediated behavioral functions at exposure levels that do not produce obvious structural changes or weight reductions in androgen-sensitive reproductive organs."
- Induction of PGE2 by estradiol mediates developmental masculinization of sex behaviour
    - "Adult male sexual behavior in mammals requires the neuronal organizing effects of gonadal steroids during a sensitive perinatal period. During development, estradiol differentiates the rat preoptic area (POA), an essential brain region in the male copulatory circuit. Here we report that increases in prostaglandin-E2 (PGE2), resulting from changes in cyclooxygenase-2 (COX-2) regulation induced by perinatal exposure to estradiol, are necessary and sufficient to organize the crucial neural substrate that mediates male sexual behavior. Briefly preventing prostaglandin synthesis in newborn males with the

COX inhibitor indomethacin permanently downregulates markers of dendritic spines in the POA and severely impairs male sexual behavior. Developmental exposure to the COX inhibitor aspirin results in mild impairment of sexual behavior. Conversely, administration of PGE2 to newborn females masculinizes the POA and leads to male sex behavior in adults, thereby highlighting the pathway of steroid-independent brain masculinization. Our findings show that PGE2functions as a downstream effector of estradiol to permanently masculinize the brain."

- A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits
    - "We tested the hypothesis that genes encoded on the sex chromosomes play a direct role in sexual differentiation of brain and behavior. We used mice in which the testis-determining gene (Sry) was moved from the Y chromosome to an autosome (by deletion of Sry from the Y and subsequent insertion of an Sry transgene onto an autosome), so that the determination of testis development occurred independently of the complement of X or Y chromosomes. We compared XX and XY mice with ovaries (females) and XX and XY mice with testes (males). These comparisons allowed us to assess the effect of sex chromosome complement (XX vs XY) independent of gonadal status (testes vs ovaries) on sexually dimorphic neural and behavioral phenotypes. The phenotypes included measures of male copulatory behavior, social exploration behavior, and sexually dimorphic neuroanatomical structures in the septum, hypothalamus, and lumbar spinal cord. Most of the sexually dimorphic phenotypes correlated with the presence of ovaries or testes and therefore reflect the hormonal output of the gonads. We found, however, that both male and female mice with XY sex chromosomes were more masculine than XX mice in the density of vasopressin-immunoreactive fibers in the lateral septum. Moreover, two male groups differing only in the form of their Sry gene showed differences in behavior. The results show that sex chromosome genes contribute directly to the development of a sex difference in the brain."
- Androgen imprinting of the brain in animal models and humans with intersex disorders: review and recommendations
    - "Animal studies support a role for postnatal androgens in brain/behavior development with human studies neither completely supportive nor antagonistic. Therefore, gender assignment in infants with intersex should be made with the possibility in mind that postnatal testicular hormones at ages 1 to 6 months may affect gender identity. A case-control study is required to test the hypothesis that postnatal androgen exposure may convert ambisexual brain functions to committed male behavior patterns."
- Sex differences in response to children's toys in nonhuman primates (Cercopithecus aethiops sabaeus)
    - "Sex differences in children's toy preferences are thought by many to arise from gender socialization. However, evidence from patients with endocrine disorders suggests that biological factors during early development (e.g., levels of androgens) are influential. In this study, we found that vervet monkeys (*Cercopithecus aethiops*sabaeus) show sex differences in toy preferences similar to those documented previously in children. The percent of contact time with toys typically preferred by boys (a car and a ball) was greater in male vervets (n=33) than in female vervets (n=30) (P<.05), whereas the percent of contact time with toys typically preferred by girls (a doll and a pot) was greater in female vervets than in male vervets (P<.01). In contrast, contact time with toys preferred equally

by boys and girls (a picture book and a stuffed dog) was comparable in male and female vervets. The results suggest that sexually differentiated object preferences arose early in human evolution, prior to the emergence of a distinct hominid lineage. This implies that sexually dimorphic preferences for features (e.g., color, shape, movement) may have evolved from differential selection pressures based on the different behavioral roles of males and females, and that evolved object feature preferences may contribute to present day sexually dimorphic toy preferences in children."

- Wired for Reproduction: Organization and Development of Sexually Dimorphic Circuits in the Mammalian Forebrain
    - "Mammalian reproduction depends on the coordinated expression of behavior with precisely timed physiological events that are fundamentally different in males and females. An improved understanding of the neuroanatomical relationships between sexually dimorphic parts of the forebrain has contributed to a significant paradigm shift in how functional neural systems are approached experimentally. This review focuses on the organization of interconnected limbic-hypothalamic pathways that participate in the neural control of reproduction and summarizes what is known about the developmental neurobiology of these pathways. Sex steroid hormones such as estrogen and testosterone have much in common with neurotrophins and regulate cell death, neuronal migration, neurogenesis, and neurotransmitter plasticity. In addition, these hormones direct formation of sexually dimorphic circuits by influencing axonal guidance and synaptogenesis. The signaling events underlying the developmental activities of sex steroids involve interactions between nuclear hormone receptors and other transcriptional regulators, as well as interactions at multiple levels with neurotrophin and neurotransmitter signal transduction pathways."
- Changes in sexual behavior of adult male and female rats neonatally treated with vitamin D3
    - "Neonatal treatment of rats with vitamin D_3 resulted in a change of sexual behavior in adulthood. 2.5 mg vitamin D_3 completely inhibited the ejaculation of males without any apparent influence on sexual desire. 250 mg vitamin D_3 influenced both the desire and ejaculation. Sexual activity of females was depressed by both doses. The experiments demonstrate that vitamin D_3, a steroid in structure, given in the critical period of hormonal imprinting may influence steroid hormone-receptor commanded events for life, in a way similar to the effects exhibited by synthetic steroid hormone analogues and benzpyrene in earlier studies."
- The pre- and postnatal influence of hormones and neurotransmitters on sexual differentiation of the mammalian hypothalamus
    - "A number of brain structures and a great number of brain functions have been shown to be sexually dimorphic. It has also been shown that development and differentiation of these structures and functions proceeds during a critical pre- and postnatal period of increased susceptibility, and is controlled by gonadal steroids and neurotransmitter substances. The brain of male and female mammals seems to be still undifferentiated before the period of increased susceptibility to gonadal steroids and neurotransmitters starts. Feminization of brain structure and functions, e.g., establishment of the cyclic LH-surge mechanism and the expression of lordosis behavior, seems to depend on the moderate interaction of estrogens with the developing nervous system. Defeminization and masculinization of brain functions seem to be established during interaction of the

developing nervous system with androgens, which have to be converted, at least in part, into estrogens. Structural differentiation of the male brain, e.g., the sexually dimorphic nucleus of the preoptic area (SDN-POA), seems to be exclusively estrogen-dependent, during differentiation of male brain functions, however, estrogens may be supportive, rather than directive, to the primary action of androgens. The molecular mechanisms of sexual differentiation of the brain are not yet fully understood. It seems, however, that the priming action of gonadal steroids during the period of increased susceptibility is either mediated by neurotransmitters, or neurotransmitters modulate the priming action of gonadal steroids. In particular, the adrenergic, the serotoninergic, the cholinergic, and possibly the dopaminergic system were shown to have strong influences on sexual differentiation of brain structure and functions. In contrast to the great number of available studies on the influence of gonadal steroids on sexual differentiation of the brain, there are rather few studies available concerning the influence of neurotransmitter systems. The available results are partly contradictory, so that an interpretation must be done with caution and will leave plenty of room for speculation. Postnatal application of compounds which stimulate or inhibit adrenergic activity mainly affected the neural control of gonadotropin secretion, and had only minor influences on differentiation of behavior patterns. It seems, however, that adrenergic participation in the differentiation of the center for cyclic gonadotropin release is very complex and stimulatory and inhibitory components may operate simultaneously. Activation or inhibition of beta-adrenergic receptors during postnatal development was shown to impair the responsiveness of the center for cyclic gonadotropin release to gonadal steroids, and impairs the expression of ejaculatory behavior in male rats."

- Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques
    - "Genetic female fetuses were exposed transplacentally to testosterone propionate injected into their mothers either early (Days 40 through 64) or late (Days 115 through 139) in gestation. Early and late androgenized females (EAFs and LAFs, respectively) were raised with normal males and females that served as criteria for evaluating degree of behavioral masculinization induced by the prenatal androgen. EAFs were genitally virilized and LAFs were not. Males and untreated females differed reliably on five behavioral measures: males showed more mother-mounting, more peer-mounting, more rough play with peers, a preference for initiating play with male partners, and less grooming of mothers. Neither type of prenatally androgenized female showed masculinization of all five types of behavior. Compared with females, EAFs showed more mother-mounting, more peer-mounting, less mother-grooming, did not differ from females in rough play, and did not manifest a preference for male partners. LAFs, like females, groomed but did not mount their mothers, and did not show a preference for male partners; but unlike females they showed elevated rough play and mounting with peers. EAFs showed a statistically significant delay in puberty onset (menarche), but LAFs did not. Mothers inspected genitalia of their offspring more often if they were males than if they were females. Mothers of EAFs inspected their offspring's genitalia as often as mothers of males, but mothers of LAFs did not. No aspect of maternal behavior was associated with either the amount or kind of masculine behavior shown toward peers. We interpret the results to mean that genital virilization is independent of, and largely irrelevant to, the expression of those behavioral traits that characterize the juvenile male

social role. Moreover, the individual behavior traits that are components of the juvenile male role are independently regulated by the organizing actions of androgen and have separable critical periods. Of the two major traits, mounting peers and rough play with peers, the latter has a greater requirement for androgenic stimulation late in prenatal life."

- Social play soliciting by male and female juvenile rats: effects of neonatal androgenization and sex of cagemates
    - "Male and female juvenile rats were individually exposed to nonplayful juvenile social stimuli in a novel test of play-soliciting behavior to examine hormonal and experiential determinants of sex differences. In Experiment 1, neonatally androgenized females engaged in play soliciting at a level equal to that of male controls and greater than that of nonandrogenized female controls. In Experiment 2, males and females were reared in unisexual and bisexual groups in order to compare long-term sex-related social experience effects on juvenile play soliciting. Males exposed only to other young males engaged in greater play soliciting than males exposed to both sexes; females, in contrast, were unaffected by sex of cagemates. Within rearing conditions, however, males engaged in greater play soliciting than females. The combined results suggest that perinatal gonadal androgen exposure effects on social play are prepotent and contribute essentially to sex differences in the initiation of social play behavior."
- Testosterone implants into the amygdala during the neonatal period masculinize the social play of juvenile female rats
    - "The masculinization of social play behavior in the rat is dependent upon the actions of androgens during the neonatal period. The amygdala, a major androgen-target region in the rat limbic brain, appears to be a critical site for this androgenic effect. We tested this hypothesis by implanting testosterone-bearing cannulae into the amygdala of female rat pups on Day 1 of life; the implants were removed on Day 8 of life. The animals were then observed daily between Days 26 and 40 of life and the frequency of play-fighting was recorded. Testosterone-implanted females, like normal males, engaged in significantly more play-fighting than did control females (implanted with cholesterol-bearing cannulae). We have also presented data indicating that the testosterone diffusion from the cannulae was, for the most part, restricted to the amygdala. Thus, testosterone implanted into the amygdala mimicked the effects previously reported for systemic testosterone injections, supporting the idea that the amygdala is a critical region for the actions of androgens on the sexual differentiation of social play behavior in the rat."
- The influence of thermal signals during embryonic development on intrasexual and sexually dimorphic gene expression and circulating steroid hormones in American alligator hatchlings (Alligator mississippiensis)
    - "Incubation temperatures experienced by developing embryos exert powerful influences over gonadal sex determination and differentiation in many species. However, the molecular mechanisms controlling these impacts remain largely unknown. We utilize the American alligator to investigate the sensitivity of the reproductive system to thermal signals experienced during development and ask specifically whether individuals of the same sex, yet derived from different incubation temperatures display persistent variation in the expression patterns of sex biased transcripts and plasma sex hormones. Our analysis focuses on assessments of circulating sex steroids and transcript abundance in brain and gonad, two tissues that display sexually dimorphic gene expression and directly contribute to diverse sexually dimorphic phenotypes. Whereas our results identify

sexually dimorphic patterns for several target gonadal genes in postnatal alligators, sex linked variation in circulating 17β-estradiol, testosterone, and expression of two brain transcripts (aromatase and gonadotropin releasing hormone) was not observed. Regarding intrasexual variation, we found that AMH transcript abundance in hatchling testes is positively correlated with temperatures experienced during sexual differentiation. We also describe highly variable patterns of gene expression and circulating hormones within each sex that are not explained by the intensity of embryonic incubation temperatures. The magnitude of sexually dimorphic gene expression, however, is directly associated with temperature for SOX9 and AMH, two transcripts with upstream roles in Sertoli cell differentiation. Collectively, our findings regarding temperature linked variation provide new insights regarding the connections between embryonic environment and persistent impacts on sexual differentiation in a reptile species that displays temperature dependent sex determination."

- Sexually dimorphic microRNA expression during chicken embryonic gonadal development
    - "MicroRNAs are a highly conserved class of small RNAs that function in a sequence-specific manner to posttranscriptionally regulate gene expression. Tissue-specific miRNA expression studies have discovered numerous functions for miRNAs in various aspects of embryogenesis, but a role for miRNAs in gonadal development and sex differentiation has not yet been reported. Using the chicken embryo as a model, microarrays were used to profile the expression of chicken miRNAs prior to, during, and after the time of gonadal sex differentiation (Embryonic Day 5.5 [E5.5], E6.5, and E9.5). Sexually dimorphic miRNAs were identified, and the expression patterns of several were subjected to further validation by in situ hybridization and Northern blot analysis. Expression of one chicken miRNA, MIR202*, was observed to be sexually dimorphic, with upregulation in the developing testis from the onset of sexual differentiation. Additional data from deep sequencing of male and female embryonic gonad RNA samples also indicated upregulation of MIR202* in male gonads. These findings provide the first evidence of sexually dimorphic miRNA expression during vertebrate gonadal sex differentiation and suggest that MIR202* may function in regulating testicular development."
- Sexual differentiation in the developing mouse brain: contributions of sex chromosome genes
    - "Neural sexual differentiation begins during embryogenesis and continues after birth for a variable amount of time depending on the species and brain region. Because gonadal hormones were the first factors identified in neural sexual differentiation, their role in this process has eclipsed investigation of other factors. Here, we use a mouse with a spontaneous translocation that produces four different unique sets of sex chromosomes. Each genotype has one normal X-chromosome and a unique second sex chromosome creating the following genotypes: XY(*x) , XX, XY(*) , XX(Y) (*) . This Y(*) mouse line is used by several laboratories to study two human aneuploid conditions: Turner and Klinefelter syndromes. As sex chromosome number affects behavior and brain morphology, we surveyed brain gene expression at embryonic days 11.5 and 18.5 to isolate X-chromosome dose effects in the developing brain as possible mechanistic changes underlying the phenotypes. We compared gene expression differences between gonadal males and females as well as individuals with one vs. two X-chromosomes. We

present data showing, in addition to genes reported to escape X-inactivation, a number of autosomal genes are differentially expressed between the sexes and in mice with different numbers of X-chromosomes. Based on our results, we can now identify the genes present in the region around the chromosomal break point that produces the Y(*) model. Our results also indicate an interaction between gonadal development and sex chromosome number that could further elucidate the role of sex chromosome genes and hormones in the sexual differentiation of behavior."

- Sex differences in Japanese macaques (Macaca fuscata): effects of prenatal testosterone on juvenile social behavior
    - "The aim of this study was to assess, in a nonhuman primate, the extent to which exposure to androgen during the prenatal period interacts with early social experience to affect the display of male or female patterns of behavior. Pregnant females from a large age-graded, heterosexual group of Japanese macaques (Macaca fuscata) were implanted about the 40th day of gestation with Silastic packets of testosterone. The packets were removed on the 100th day of gestation, and the females were allowed to give birth in their outdoor corral. An unplanned procedural change, by the surgeon who did the implants, created two groups of prenatally androgenized females: a high-dose group (N = 3), and a low-dose group (N = 4). The anatomical differentiation of these groups differed in that the high-dose group had small penises and no vaginas while the low-dose group had enlarged clitorises and patent vaginas. The behavior of these two groups of females was compared with that of normal males (N = 6), prenatally androgenized males (N = 6), and normal females (N = 5) from birth to 2 years of age. There were no differences between treated and normal males, but there were sex differences between males and normal females in the frequency of mounting, playing, displaying, and grooming. The high-dose group of prenatally androgenized females differed from normal females on only one measure: increased frequency of mounting. The low-dose group mounted other juveniles more frequently than did the normal females, but the difference was not statistically significant. We concluded that mounting behavior was most sensitive to the prenatal hormone environment because it showed the largest sex difference in normal animals. Given the small sample sizes, within-group variability could have obscured possible hormonal effects on other behaviors where sex differences were less dramatic."
- Female sexual behavior displayed by androgenized female rhesus macaques
    - "The aim of this study was to assess, in a nonhuman primate, the extent to which exposure to androgen during the prenatal period interacts with early social experience to affect the display of male or female patterns of behavior. Pregnant females from a large age-graded, heterosexual group of Japanese macaques (Macaca fuscata) were implanted about the 40th day of gestation with Silastic packets of testosterone. The packets were removed on the 100th day of gestation, and the females were allowed to give birth in their outdoor corral. An unplanned procedural change, by the surgeon who did the implants, created two groups of prenatally androgenized females: a high-dose group (N = 3), and a low-dose group (N = 4). The anatomical differentiation of these groups differed in that the high-dose group had small penises and no vaginas while the low-dose group had enlarged clitorises and patent vaginas. The behavior of these two groups of females was compared with that of normal males (N = 6), prenatally androgenized males (N = 6), and normal females (N = 5) from birth to 2 years of age.

There were no differences between treated and normal males, but there were sex differences between males and normal females in the frequency of mounting, playing, displaying, and grooming. The high-dose group of prenatally androgenized females differed from normal females on only one measure: increased frequency of mounting. The low-dose group mounted other juveniles more frequently than did the normal females, but the difference was not statistically significant. We concluded that mounting behavior was most sensitive to the prenatal hormone environment because it showed the largest sex difference in normal animals. Given the small sample sizes, within-group variability could have obscured possible hormonal effects on other behaviors where sex differences were less dramatic."

- Sexual behavior in adult gonadectomized female pseudohermaphrodite, female, and male rhesus macaques (Macaca mulatta) treated with estradiol benzoate and testosterone propionate
    - "The aim of this study was to assess, in a nonhuman primate, the extent to which exposure to androgen during the prenatal period interacts with early social experience to affect the display of male or female patterns of behavior. Pregnant females from a large age-graded, heterosexual group of Japanese macaques (Macaca fuscata) were implanted about the 40th day of gestation with Silastic packets of testosterone. The packets were removed on the 100th day of gestation, and the females were allowed to give birth in their outdoor corral. An unplanned procedural change, by the surgeon who did the implants, created two groups of prenatally androgenized females: a high-dose group (N = 3), and a low-dose group (N = 4). The anatomical differentiation of these groups differed in that the high-dose group had small penises and no vaginas while the low-dose group had enlarged clitorises and patent vaginas. The behavior of these two groups of females was compared with that of normal males (N = 6), prenatally androgenized males (N = 6), and normal females (N = 5) from birth to 2 years of age. There were no differences between treated and normal males, but there were sex differences between males and normal females in the frequency of mounting, playing, displaying, and grooming. The high-dose group of prenatally androgenized females differed from normal females on only one measure: increased frequency of mounting. The low-dose group mounted other juveniles more frequently than did the normal females, but the difference was not statistically significant. We concluded that mounting behavior was most sensitive to the prenatal hormone environment because it showed the largest sex difference in normal animals. Given the small sample sizes, within-group variability could have obscured possible hormonal effects on other behaviors where sex differences were less dramatic."
- Pre- and postnatal oral toxicity of vinclozolin in Wistar and Long-Evans rats
    - "Vinclozolin administered to pregnant Wistar and Long-Evans rats from day 14 postcoitum to day 3 postpartum at 200 mg/kg body wt/day was maternally toxic (reduced food consumption and body weight gain) and increased perinatal mortality; major adverse effects on sex-specific organs in male offspring were seen (reduced anogenital distance and index; persistence of nipples/areolas into adulthood; hypospadic penis; penile hypoplasia or development of a vaginal pouch; transient paraphimosis; hypoplasia and chronic inflammation of epididymides, prostate, seminal vesicles, and coagulating glands; and also testicular tubular atrophy and chronic inflammation of the urinary bladder in some Long-Evans) with isolated inflammation-related deaths due to

pyelonephritis. At 12 mg/kg, prevalence of female areola/nipple anlagen in immature (preweaning) male offspring was increased in both strains; these persisted to adulthood in a few treated Long-Evans but not Wistar offspring. Adult Long-Evans but not Wistar at this dose also had hypoplasia of prostate, seminal vesicles, and coagulating glands, and a minority had testicular tubular atrophy. The no-observed-adverse-effect levels (NOAEL) were 12 and 6 mg/kg body wt in Wistar and Long-Evans rats, respectively, in these studies. The data suggest that both the Long-Evans and the Wistar rats are comparably sensitive to the antiandrogenic effects of vinclozolin. At dose levels below the NOAEL (1 and 3 mg/kg, respectively), there were no indications of any test-substance-related effects."

- Exposure of juvenile guppies to three antiandrogens causes demasculinization and a reduced sperm count in adult males
    - "It has been thoroughly established that the fungicide vinclozolin and the persistent DDT metabolite p,p'-DDE, can function as antiandrogens in mammals in a manner similar to the therapeutic antiandrogen flutamide. In mammals, these chemicals bind the androgen receptor and prevent the transcription of the associated genes causing abnormal sexual development and demasculinization. There are few similar studies in fish and so far it has not been demonstrated that these chemicals have any antiandrogenic effects in this group. In the present study, juvenile guppies (Poecilia reticulata) were fed sublethal doses of vinclozolin, p,p'-DDE or flutamide from birth to adulthood. At sexual maturity, we measured a suite of male sexual characteristics that are known to be under androgen control. All three chemicals caused a reduction in the orange display coloration, inhibited gonopodium development, reduced the sperm count and suppressed courtship behaviour, in a manner consistent with antiandrogen action. Only the gonodosomatic index was unaffected by the treatments. In addition, the three chemicals skewed the sex ratio at adulthood and caused delayed maturation. The altered characters are all considered to be important for male mating success and their impairment indicates that antiandrogens may seriously compromise male reproductive fitness."
- Sexual differentiation of social play in rat pups is mediated by the neonatal androgen-receptor system
    - "Social play in juvenile rats, like that in several primate species (including humans), is sexually dimorphic. Males initiate and become involved in more play-fights than do their female peers. The play-fighting of female pups can be masculinized by neonatal exposure to either testosterone or 5α-dihydrotestosterone, suggesting that in the rat the masculinization of social play is mediated by the androgen-receptor system. In this paper we present evidence that further supports this hypothesis. In the first study we found that male pups treated with the antiandrogen, flutamide, during the neonatal period (days 1 through 10 of life) engaged in less play-fighting than did control males. Moreover, flutamide-treated, male pups play-fought no more frequently than did untreated females. In a subsequent study we found that flutamide, which blocked the masculinization of social play, dramatically reduced a testosterone-induced translocation of androgen receptors to the nuclear compartment in various brain regions (as measured by a nuclear exchange assay) in neonates. In a final study we examined the play-fighting of juvenile rat pups bearing the testicular feminization mutation (Tfm); animals that are known to be insensitive to androgens and show a marked deficiency of androgen receptors. Tfm males engaged in significantly less play-fighting than did control males. The Tfm males,

like the flutamide-treated males, engaged in play-fighting at rates that were comparable to those of females. Taken together with previous data these findings strongly suggest that the sexual differentiation of social play in juvenile rats is mediated by the androgen-receptor system during the neonatal period."

- Prenatal stress feminizes juvenile play patterns in male rats
    - "Sexually dimorphic rough-and-tumble play patterns were compared in male and female rats derived from control mothers and mothers stressed from days 14-21 of pregnancy. Animals were weaned into groups of 8 consisting of 2 males and 2 females from each treatment. Play in the home cage was recorded at 25, 28, 31, 34, 37 and 45 days of age and was most intense on day 31. The overall level of play was significantly higher in control males than in females or stressed males. Control males showed higher levels of the pinning component of rough-and-tumble play than females or stressed males. No play partner preferences were detected in any group. In adulthood, a higher percentage of stressed than control males displayed the female lordotic pattern. No deficits in ejaculatory behavior occurred in the stressed males. Since maternal stress alters patterns of plasma testosterone in male fetuses, the data suggest that the sexual differentiation of social play begins during prenatal ontogeny in the rat. The present results show that sexually dimorphic behaviors displayed before puberty are incompletely masculinized in prenatally stressed males, a finding similar to that reported for a number of adult behaviors."
- Spatial memory performance in androgen insensitive male rats
    - "Masculinization of the developing rodent brain critically depends on the process of aromatization of circulating testosterone (T) to its estrogenic metabolite 17beta-estradiol, which subsequently interacts with estrogen receptors to permanently masculinize the brain. However, it remains unclear what role other androgenic mechanisms may play in the process of masculinization. A novel way of examining this is through the study of male rats that express the tfm mutation of the androgen receptor (AR) gene; such males are fully androgen insensitive and manifest a female phenotype due to a failure of AR-mediated masculinization of peripheral structures. Because tfm-affected males develop secretory testes and have near-normal T titers during development, aromatization would be expected to proceed normally, and brain mechanisms may be developmentally masculinized despite the feminized periphery. We compared tfm-affected males (X(tfm)Y) with normal males and females in the Morris Water Maze, a task in which males typically perform better than females. Performance of tfm-affected males was intermediate between that of normal males and females. While an overall male superiority was found in the task, the X(tfm)Y group reached male-typical escape latencies faster than females. Furthermore, in the X(tfm)Y group, the granule cell layer of the dentate gyrus was significantly larger than in females. These results support the suggestion that that AR mediated mechanisms contribute to the masculinization of spatial behaviours and hippocampal morphology, and this may be independent of estrogenic processes."
- Evidence for a morphological sex difference within the medial preoptic area of the rat brain
    - "The present report demonstrates the existence of a marked sexual difference in the volume of an intensely staining cellular component of the medial preoptic nucleus (MPON) of the rat. Moreover, this sexual dimorphism is shown to be independent of

several specific hormonal conditions in the adult, but significantly influenced, perhaps determined, by the perinatal hormone environment. Adult rats were gonadectomized and sacrificed 2 or 5-6 weeks later, or sacrificed after gonadectomy and priming with estradiol benzoate (2 microgram/day x 3) and 500 microgram progesterone, or testosterone propionate (TP, 500 microgram/day x 14), or the ingestion of propylthiouracil (0.15% of the diet) for one month, or following water deprivation for 24 h. These treatments did not affect the sexual dimorphism in the MPON and, in all groups, nuclear volume in the male animals was significantly greater than that of females whether nuclear volume was expressed in absolute terms or relative to brain weight. On the other hand, the volume of the MPON of the adult male castrated neonatally was significantly reduced when compared to that of the male castrated at the time of weaning, i.e. after the period of sexual differentiation of the brain. Consistent with the view that this nuclear region undergoes sexual differentiation is the fact that the volume of the MPON was significantly greater in female rats injected with 1 mg TP on day 4 of life than in oil-treated females. More subtle sex differences in the volume of the suprachiasmatic nucleus were also detected, as were several treatment effects. Although these differences may fall within the error of the analytical procedure, it is possible that hormone- or sex-dependent morphological differences exist elsewhere in the brain. Nevertheless, the gross sexual dimorphism in the MPON clearly demonstrates a possible morphological basis for the sexual differentiation of brain function"

- Differential effects of neonatal castration on the development of sexually dimorphic brain areas in the gerbil
    - "We compared the effects of neonatal castration within 6 h of birth in the Mongolian gerbil on the development of the sexually dimorphic area pars compacta (SDApc) and supraschiasmatic nucleus (SCN). Development of these brain areas was also related to a masculine courtship ultrasonic vocalization, the frequency-modulated upsweep. Castration immediately after birth resulted in differential effects with complete or partial reduction of SCN and SDApc volumes, respectively, as compared to male values. The rates of ultrasonic calling of males castrated as neonates were also decreased to female levels. In sham-operated males, calling rates were positively correlated with the volume of the left SDApc, but not the right. Both the left and the right SDApc volumes were correlated with calling rates in males castrated as neonates. The asymmetric relationship between vocal behavior and area volume was specific to the SDApc. We suggest that in the neonate (a) the sensitivity of the SDApc to the differentiating effects of androgens differs from the SCN and (b) the asymmetric link between brain structure and vocal behavior depends on the effects of androgen within 6 h of birth"
- Sex difference in volume of the ventromedial nucleus of the hypothalamus in the rat
    - "The volume of the ventromedial nucleus of the hypothalamus (VMN) of normal male rats was significantly greater than that of normal female rats. Castration of day 1 neonatal males significantly reduced the volume of the VMN to a level comparable with that of normal females. However, the VMN volume was no longer influenced by castration on day 7. Injection of 1.25 mg testosterone propionate to 5- to 15-day-old females did not have any significant effect on the volume of the VMN. These results indicate that the volume of the VMN is sexually dimorphic and is modified by internal secretion of neonatal testes"
- Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat

    - "The volume of an intensely staining component of the preoptic area of the male rat is markedly larger than that of the female. Moreover, its volume in both sexes is altered by perinatal hormone exposure consistent with the view that this brain region undergoes hormone dependent sexual differentiation. The present study was carried out to determine if this sexually dimorphic area of the brain has a greater cell density than that of the surround, and if a unique population or distribution of cells, either within one sex or between males and females, characterized this region. A single coronal paraffin section (10 micrometer) through the approximate center of this sexually dimorphic area in four adult gonadectomized rats of each sex was evaluated systematically. Each cell was labelled as being inside or outside of the sexually dimorphic area. In addition to cell density per unit area the following parameters were evaluated through a closed-circuit video system: cell size, staining intensity, shape, and the presence of processes and of a nucleolus. The presence of a nucleolus was further used to identify neurons within the total population of almost 5000 cells that was evaluated. In both sexes, the sexually dimorphic area was characterized by a significantly increased cell density per unit area compared to that of the surround. On this basis, the term, the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) is proposed, for this region. Moreover, the SDN-POA of the male was characterized by increased neuronal density per unit area. The SDN-POA in the male was also found to contain larger cells and neurons, as determined by direct measurement of their greatest diameter, as well as a greater percentage of cells and neurons rated large on a three-point scale (small, medium, and large). No consistent differences in frequency distribution by stain intensity, shape, or the presence of cell processes were found to characterize the SDN-POA or contribute to the sexual dimorphism. It is concluded that the marked sex difference in the volume of the SDN-POA is due principally to an increase in the male of the total area of higher cell and neuronal density. However, the present results do not eliminate the possibility that more subtle differences in neuronal characteristics may exist in the SDN-POA."
- Organizational effects of early gonadal secretions on sexual differentiation in spatial memory
    - "Neonatally castrated (MNC) and control male rats (MC) and female rats treated neonatally with estradiol benzoate (FNE) and female controls (FC) were studied. In Exp. 1 spatial memory was assessed using a 12-arm radial maze. During acquisition, MC and FNE groups were more accurate in choice behavior than FC and MNC groups. In Exp. 2 the discriminative control exerted by different types of cues was evaluated. Alteration of the geometry of the room but not movable landmarks disrupted performance of MC and FNE groups. For the FC and MNC groups, alteration of either geometry or landmarks did not disrupt performance. In Exp. 3 the effect of a 15-min delay was determined. MC and FNE groups were more disrupted by a delay than MNC and FC groups. Together, these data suggest that early exposure to gonadal steroids (probably estradiol) improves acquisition of spatial tasks by reorganizing and simplifying associational-perceptual processes that guide spatial ability."
- Testosterone and estradiol produce different effects on cognitive performance in male rats
    - "The effects of castration and hormone treatment on cognitive performance were evaluated in male rats. Castrated animals received either testosterone or estradiol and were compared with gonadally intact animals and with castrated controls. Results

revealed a dissociation between the effects of testosterone and estradiol on cognitive performance in male rats. Specifically, estradiol enhanced acquisition of a delayed matching-to-position spatial task, similar to previously published observations in females. In contrast, neither castration nor testosterone treatment had any significant effect on acquisition of the delayed matching-to-position task, but did appear to affect delay-dependent working memory. None of the treatments had any significant effect on acquisition of a configural association negative patterning task, suggesting that effects on the delayed matching-to-position task were not due to effects on motivational factors. These data demonstrate that, as in females, gonadal hormones influence cognitive performance in males and suggest that estradiol and testosterone affect distinct cognitive domains."

- Testosterone modulates performance on a spatial working memory task in male rats
    - "Gonadal hormones have been shown to modulate memory retention in female rats. The current experiments examine the role of testicular hormones in modulating the performance of male rats on two spatial water maze tasks. In the first study, castrated and intact rats were trained on the visible platform and hidden platform versions of the Morris water maze task. Castration did not affect performance on either version of this reference memory task with castrated and intact rats demonstrating similar performance both during acquisition and on post-training probe trials. In the second experiment, castrated and intact rats were tested on a delayed-matching-to-place version of the water maze. Rats received a series of trial pairs in the maze with a hidden platform located in the same pool location on the exposure and retention trials of each pair; between pairs of trials, however, the platform was repositioned to a novel pool location. The interval between trials was either 10- or 60-min and memory retention, taken as the difference between the pathlengths on the exposure and retention trials, declined as the interval increased. Relative to intact males, castrated males demonstrated impaired working memory retention at 60-min but not at 10-min retention intervals. This interval-dependent impairment in working memory retention was reversed by physiologic levels of testosterone replacement. These findings indicate that castration does not significantly affect acquisition or probe trial performance on a classic reference memory task but does impair spatial working memory retention, an effect that is reversed by exogenous testosterone."
- Sex differences in the play behavior of prepubescent rats
    - "Male and female rat pups received subcutaneous injections of 500 µg testosterone propionate in 0.05 ml sesame oil at 24 and 48 hr of age. Oil-treated animals received comparable injections of sesame oil alone. Social behavior was observed in same-sex, same-treatment pairs during brief encounters at 22, 26, 30 and 40 days of age. Oil-treated males spent significantly more time in rough-and-tumble play than did oil-treated females. Testosterone treatment during postnatal life significantly increased the time spent in play by females to levels comparable to those of oil-treated males. The results demonstrate that in the rat, as in primates, perinatal exposure to testicular androgens influences the development of patterns of play behavior."
- Differential rates of attack, defense, and counterattack during the developmental decrease in play fighting by male and female rats
    - "During postweaning development, rats exhibit several well documented trends in their play fighting: (1) It peaks between 30-40 days and then declines with the approach of

sexual maturity; (2) males initiate more play fights than females; and (3) the overall complexity of play fights, as expressed by such measures as duration of bouts, also decreases with increasing age. Such trends could arise from changes in attack or defense, or some combination of both. In this article it is shown that (a) the decline in play fighting with the onset of sexual maturity in rats results from a decline in attack, not in defense; (b) the differences in play fighting by male and female rats are due to sex-specific rates of both attack and defense; and (c) the developmental decrease in the complexity of play fighting arises from a decrease in the frequency of counterattacks (i.e., after an animal defends itself, it is less likely to launch an attack). In this way, age and sex differences in play fighting can be traced to differences in its subcomponents."

- Behavioral effects of estrogen receptor gene disruption in male mice
    - "Gonadal steroid hormones regulate sexually dimorphic development of brain functions and behaviors. Their nuclear receptors offer the opportunity to relate molecular events in neurons to simple instinctive mammalian behaviors. We have determined the role of estrogen receptor (ER) activation by endogenous estrogen in the development of male-typical behaviors by the use of transgenic estrogen-receptor-deficient (ERKO) mice. Surprisingly, in spite of the fact that they are infertile, ERKO mice showed normal motivation to mount females but they achieved less intromissions and virtually no ejaculations. Aggressive behaviors were dramatically reduced and male-typical offensive attacks were rarely displayed by ERKO males. Moreover, ER gene disruption demasculinized open-field behaviors. In the brain, despite the evident loss of functional ER protein, the androgen-dependent system appears to be normally present in ERKO mice. Together, these findings indicate that ER gene expression during development plays a major role in the organization of male-typical aggressive and emotional behaviors in addition to simple sexual behaviors."
- Is feminine differentiation of the brain hormonally determined?
    - "The androgen insensitive, genetically male rat pseudohermaphrodite displays neither masculine or feminine sexual behavior when primed with the appropriate sex hormones. Although in the absence of androgen imprinting the animal develops anatomically as female, our results suggest that feminine differentiation of the brain requires active imprinting by perinatal hormone(s), possibly adrenal progesterone."
- Sex chromosome genes directly affect brain sexual differentiation
    - "Sex differences in the brain are caused by differences in gonadal secretions: higher levels of testosterone during fetal and neonatal life cause the male brain to develop differently than the female brain. In contrast, genes encoded on the sex chromosomes are not thought to contribute directly to sex differences in brain development, even though male (XY) cells express Y-chromosome genes that are not present in female (XX) cells, and XX cells may have a higher dose of some X-chromosome genes. Using mice in which the genetic sex of the brain (XX versus XY) was independent of gonadal phenotype (testes versus ovaries), we found that XY and XX brain cells differed in phenotype, indicating that a brain cell's complement of sex chromosomes may contribute to its sexual differentiation."

Genetic Factors and Transsexualism

Some previously-listed citations have been repeated, as they are relevant to this category as well.

Human Studies

- Early androgen exposure and human gender development
    - "During early development, testosterone plays an important role in sexual differentiation of the mammalian brain and has enduring influences on behavior. Testosterone exerts these influences at times when the testes are active, as evidenced by higher concentrations of testosterone in developing male than in developing female animals. This article critically reviews the available evidence regarding influences of testosterone on human gender-related development. In humans, testosterone is elevated in males from about weeks 8 to 24 of gestation and then again during early postnatal development. Individuals exposed to atypical concentrations of testosterone or other androgenic hormones prenatally, for example, because of genetic conditions or because their mothers were prescribed hormones during pregnancy, have been consistently found to show increased male-typical juvenile play behavior, alterations in sexual orientation and gender identity (the sense of self as male or female), and increased tendencies to engage in physically aggressive behavior. Studies of other behavioral outcomes following dramatic androgen abnormality prenatally are either too small in their numbers or too inconsistent in their results, to provide similarly conclusive evidence. Studies relating normal variability in testosterone prenatally to subsequent gender-related behavior have produced largely inconsistent results or have yet to be independently replicated. For studies of prenatal exposures in typically developing individuals, testosterone has been measured in single samples of maternal blood or amniotic fluid. These techniques may not be sufficiently powerful to consistently detect influences of testosterone on behavior, particularly in the relatively small samples that have generally been studied. The postnatal surge in testosterone in male infants, sometimes called mini-puberty, may provide a more accessible opportunity for measuring early androgen exposure during typical development. This approach has recently begun to be used, with some promising results relating testosterone during the first few months of postnatal life to later gender-typical play behavior. In replicating and extending these findings, it may be important to assess testosterone when it is maximal (months 1 to 2 postnatal) and to take advantage of the increased reliability afforded by repeated sampling."
- Androgen receptor function links human sexual dimorphism to DNA methylation
    - "Sex differences are well known to be determinants of development, health and disease. Epigenetic mechanisms are also known to differ between men and women through X-inactivation in females. We hypothesized that epigenetic sex differences may also result from sex hormone functions, in particular from long-lasting androgen programming. We aimed at investigating whether inactivation of the androgen receptor, the key regulator of normal male sex development, is associated with differences of the patterns of DNA methylation marks in genital tissues. To this end, we performed large scale array-based analysis of gene methylation profiles on genomic DNA from labioscrotal skin fibroblasts of 8 males and 26 individuals with androgen insensitivity syndrome (AIS) due to inactivating androgen receptor gene mutations. By this approach we identified differential methylation of 167 CpG loci representing 162 unique human genes. These were significantly enriched for androgen target genes and low CpG content promoter genes. Additional 75 genes showed a significant increase of heterogeneity of methylation in AIS compared to a high homogeneity in normal male controls. Our data

show that normal and aberrant androgen receptor function is associated with distinct patterns of DNA-methylation marks in genital tissues. These findings support the concept that transcription factor binding to the DNA has an impact on the shape of the DNA methylome. These data which derived from a rare human model suggest that androgen programming of methylation marks contributes to sexual dimorphism in the human which might have considerable impact on the manifestation of sex-associated phenotypes and diseases."

- Pacific Center for Sex and Society - Intersex and Transsex: Atypical Gender Development and Social Construction
    - "In summary, the behaviors of intersexed and transgendered persons provide a wide range of evidence against many aspects of social science and social construction theory. Intersexed and transgendered persons, as well as typical persons, are each born with a certain background based upon evolutionary heritage, family genetics, uterine environment, and health factors that they will evidence in a socially permissive culture and limit in a restrictive one. The strongest gestational influences are from genetic and endocrinal organizing forces. Organizing factors are those genetic and hormonal influences established prenatally that influence postnatal behaviors set in motion by social or other environmental activation processes (such as puberty) or events (such as serious threats). Organizing factors influence or bias subsequent responses of the individual to environmental/social forces; they predispose the person to manifest behaviors and attitudes (biases) that have come to be recognized as appropriate. Sex-related activation effects occur postnatally; most noticeably at or after puberty. The lives of intersex and transgendered persons provide strong evidence for a realistic theory of sexual development: biased-interaction theory."
- Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior
    - "Both sexual orientation and sex-typical childhood behaviors, such as toy, playmate and activity preferences, show substantial sex differences, as well as substantial variability within each sex. In other species, behaviors that show sex differences are typically influenced by exposure to gonadal steroids, particularly testosterone and its metabolites, during early development (prenatally or neonatally). This article reviews the evidence regarding prenatal influences of gonadal steroids on human sexual orientation, as well as sex-typed childhood behaviors that predict subsequent sexual orientation. The evidence supports a role for prenatal testosterone exposure in the development of sex-typed interests in childhood, as well as in sexual orientation in later life, at least for some individuals. It appears, however, that other factors, in addition to hormones, play an important role in determining sexual orientation. These factors have not been well-characterized, but possibilities include direct genetic effects, and effects of maternal factors during pregnancy. Although a role for hormones during early development has been established, it also appears that there may be multiple pathways to a given sexual orientation outcome and some of these pathways may not involve hormones."
- Reframing sexual differentiation of the brain
    - "In the twentieth century, the dominant model of sexual differentiation stated that genetic sex (XX versus XY) causes differentiation of the gonads, which then secrete gonadal hormones that act directly on tissues to induce sex differences in function. This serial model of sexual differentiation was simple, unifying and seductive. Recent evidence,

however, indicates that the linear model is incorrect and that sex differences arise in response to diverse sex-specific signals originating from inherent differences in the genome and involve cellular mechanisms that are specific to individual tissues or brain regions. Moreover, sex-specific effects of the environment reciprocally affect biology, sometimes profoundly, and must therefore be integrated into a realistic model of sexual differentiation. A more appropriate model is a parallel-interactive model that encompasses the roles of multiple molecular signals and pathways that differentiate males and females, including synergistic and compensatory interactions among pathways and an important role for the environment."

- Sexual differentiation of the brain related to gender identity: beyond hormones
    - "The sexual differentiation of the brain starts in the second semester of pregnancy, which is, after the development of the genitals which differentiate in the second month of pregnancy. Because these two processes have different timetables, it could be that these are initiated through different pathways. Male gonads synthesize testosterone, which can be converted into estrogen by aromatase in the brain. In humans, the exact mechanism of male and female brain development has still to be elucidated. Based on clinical evidence from genetic men (XY) suffering from a mutation in the androgen receptor gene (complete androgen-insensitivity syndrome) and who show a female phenotype of the external genitals as well as the brain, it can be proposed that direct action of testosterone is probably causing the brain to differentiate in the male direction. However, when the process of genital development and of brain sexual development does not match the same sex, females with a male brain and vice versa can arise. These transsexual people have problems with their gender identity and have the conviction of being born in the wrong body. Twin and family studies show that there are genetic factors influencing the chances of a gender identity problem. Genetic factors could play a large role in the sexual differentiation of the brain, as can be shown from studies where differential genetic expression is found before development of the gonads. These genes could also function in other tissues than gonads and influence the sexual differentiation of the brain. The DMRT gene family which encodes transcription factors or the amount of sex hormone binding globulin (SHBG) is possibly influencing the development of sex differences, just as sex-biased differential splicing. Epigenetic mechanisms such as X-inactivation and genomic imprinting are also good candidates for causing differences in the sexual differentiation of the brain. These observations indicate that probably many processes operate together in the sexual differentiation of the brain and that diverse mutations can lead to gender identity problems."
- The Epigenetics of Sex Differences in the Brain
    - "Epigenetic changes in the nervous system are emerging as a critical component of enduring effects induced by early life experience, hormonal exposure, trauma and injury, or learning and memory. Sex differences in the brain are largely determined by steroid hormone exposure during a perinatal sensitive period that alters subsequent hormonal and nonhormonal responses throughout the lifespan. Steroid receptors are members of a nuclear receptor transcription factor superfamily and recruit multiple proteins that possess enzymatic activity relevant to epigenetic changes such as acetylation and methylation. Thus steroid hormones are uniquely poised to exert epigenetic effects on the developing nervous system to dictate adult sex differences in brain and behavior. Sex differences in the methylation pattern in the promoter of estrogen and progesterone

receptor genes are evident in newborns and persist in adults but with a different pattern. Changes in response to injury and in methyl-binding proteins and steroid receptor coregulatory proteins are also reported. Many steroid-induced epigenetic changes are opportunistic and restricted to a single lifespan, but new evidence suggests endocrine-disrupting compounds can exert multigenerational effects. Similarly, maternal diet also induces transgenerational effects, but the impact is sex specific. The study of epigenetics of sex differences is in its earliest stages, with needed advances in understanding of the hormonal regulation of enzymes controlling acetylation and methylation, coregulatory proteins, transient versus stable DNA methylation patterns, and sex differences across the epigenome to fully understand sex differences in brain and behavior."

- Mecp2 Organizes Juvenile Social Behavior in a Sex-Specific Manner
    - "Methyl-CpG-binding protein 2 (MeCP2) binds methylated DNA and recruits corepressor proteins to modify chromatin and alter gene transcription. Mutations of the *MECP2* gene can cause Rett syndrome, whereas subtle reductions of MeCP2 expression may be associated with male-dominated social and neurodevelopmental disorders. We report that transiently decreased amygdala Mecp2 expression during a sensitive period of brain sexual differentiation disrupts the organization of sex differences in juvenile social play behavior. Interestingly, neonatal treatment with Mecp2 small interfering RNA within the developing amygdala reduced juvenile social play behavior in males but not females. Reduced Mecp2 expression did not change juvenile sociability or anxiety-like behavior, suggesting that this disruption is associated with subtle behavioral modification. This suggests that Mecp2 may have an overlooked role in the organization of sexually dimorphic behaviors and that male juvenile behavior is particularly sensitive to Mecp2 disruption during this period of development."
- The control of sexual differentiation of the reproductive system and brain
    - "This review summarizes current knowledge of the genetic and hormonal control of sexual differentiation of the reproductive system, brain and brain function. While the chromosomal regulation of sexual differentiation has been understood for over 60 years, the genes involved and their actions on the reproductive system and brain are still under investigation. In 1990, the predicted testicular determining factor was shown to be the SRY gene. However, this discovery has not been followed up by elucidation of the actions of SRY, which may either stimulate a cascade of downstream genes, or inhibit a suppressor gene. The number of other genes known to be involved in sexual differentiation is increasing and the way in which they may interact is discussed. The hormonal control of sexual differentiation is well-established in rodents, in which prenatal androgens masculinize the reproductive tract and perinatal oestradiol (derived from testosterone) masculinizes the brain. In humans, genetic mutations have revealed that it is probably prenatal testosterone that masculinizes both the reproductive system and the brain. Sexual differentiation of brain structures and the way in which steroids induce this differentiation, is an active research area. The multiplicity of steroid actions, which may be specific to individual cell types, demonstrates how a single hormonal regulator, e.g. oestradiol, can exert different and even opposite actions at different sites. This complexity is enhanced by the involvement of neurotransmitters as mediators of steroid hormone actions. In view of current environmental concerns, a brief summary of the effects of endocrine disruptors on sexual differentiation is presented."

- Transsexualism. Epidemiology, phenomenology, regret after surgery, aetiology, and public attitudes
    - “Transsexualism denotes a condition in which the gender identity-the personal sense of being a man or a woman-contradicts the bodily sex characteristics. This thesis is based on three independent surveys about transsexualism. FIRST, all 233 subjects applying for sex reassignment in Sweden during 1972-1992 were retrospectively examined through medical records. The incidence of applying for sex reassignment was 0.17/100,000 individuals over 15 years of age and per year. The male-to-female (M-F)/female-to-male (F-M) ratio was 1.4/1. With the exception of an incidence peak related to the legislation regulating sex reassignment in the early 1970s, the incidence has remained fairly stable since the first estimates in Sweden in the late 1960s. The M-F (n=134) and F-M (n=99) groups were phenomenologically compared. M-F transsexuals were older, and more often had a history of marriage and children than their F-M counterparts. M-F transsexuals also had more heterosexual experience. F-M transsexuals, on the other hand, more frequently reported cross-gender behaviour in childhood than did M-F transsexuals. It is concluded that transsexualism is manifested differently in males and females. The regret frequency (defined as applying for reversal to the original sex) was 3.8%. Prognostic factors for regret were, 'a poor support from the family', and 'belonging to the secondary group of transsexuals' (denotes people who develop transsexualism only after a significant period of transvestism or homosexuality). SECOND, 28 M-F transsexuals and 30 male controls were investigated. To test the hypothesis that genes coding for proteins involved in the sexual differentiation of the brain influence the susceptibility of transsexualism, we analysed (1) a tetra nucleotide polymorphism of the aromatase gene, (2) a CAG repeat sequence in the first exon of the gene coding for the androgen receptor, and (3) a CA repeat polymorphism of the estrogen receptor beta gene. Results support the notion that the gender identity is related to the sex steroid-driven sexual differentiation of the brain, and that certain genetic variants of three of the genes critically involved in this process, may enhance the susceptibility for transsexualism.THIRD, a questionnaire comprising questions about attitudes towards transsexualism and transsexuals was mailed to a random national sample (n=998) of Swedish residents, 18-75 years of age. The response rate was 67%. The results showed that a majority supports the possibility for transsexuals to undergo sex reassignment. However, 63% thought that the individual should bear the expenses for it. In addition, a majority supported the transsexuals' right to get married in their new sex, and their right to work with children. Transsexuals' right to adopt and raise children was supported by 43% whereas 41% opposed this. The results indicated that those who believed that transsexualism is caused by psychological factors had a more restrictive view on transsexualism than people who held a biological view.”
- Gene- and environment-dependent neuroendocrine etiogenesis of homosexuality and transsexualism
    - “Sexual brain organization is dependent on sex hormone and neurotransmitter levels occurring during critical developmental periods. The higher the androgen levels during brain organization, caused by genetic and/or environmental factors, the higher is the biological predisposition to bi- and homosexuality or even transsexualism in females and the lower it is in males. Adrenal androgen excess, leading to heterotypical sexual orientation and/or gender role behavior in genetic females, can be caused by

21-hydroxylase deficiency, especially when associated with prenatal stress. The cortisol (F) precursor 21-deoxycortisol (21-DOF) was found to be significantly increased after ACTH stimulation in homosexual as compared to heterosexual females. 21-DOF was increased significantly before and even highly significantly after ACTH stimulation in female-to-male transsexuals. In view of these data, heterozygous and homozygous forms, respectively, of 21-hydroxylase deficiency represent a genetic predisposition to androgen-dependent development of homosexuality and transsexualism in females. Testicular androgen deficiency in prenatal life, giving rise to heterotypical sexual orientation and/or gender role behavior in genetic males, may be induced by prenatal stress and/or maternal or fetal genetic alterations. Most recently, in mothers of homosexual men--following ACTH stimulation--a significantly increased prevalence of high 21-DOF plasma values and 21-DOF/F ratios was found, which surpassed the mean + 1 SD level of heterosexual control women. In homosexual men as well--following ACTH stimulation--most of the 21-DOF plasma values and 21-DOF/F ratios also surpassed the mean + 1 SD level of heterosexual men. In only one out of 9 homosexual males, neither in his blood nor in that of his mother increased 21-DOF values and 21-DOF/F ratios were found after ACTH stimulation. In this homosexual man, however, the plasma dehydroepiandrosterone sulfate (DHEA-S) values and the DHEA-S/1000 x A (A = androstenedione) ratio were increased before and after ACTH stimulation. Furthermore, highly significantly increased basal plasma levels of dehydroepiandrosterone sulfate were found in male-to-female transsexuals as compared to normal males, suggesting partial 3 beta-ol hydroxysteroid dehydrogenase deficiency to be a predisposing factor for the development of male-to-female transsexualism."

Animal Studies

- Epigenetically programming gender identity
    - "The presence or absence of a Y chromosome dictates our biological gender. However, the brain is wired to be female unless exposed to testicular steroids (such as androgen and its metabolite estradiol) during a sensitive period shortly before and after birth. The most sexually dimorphic region in the brain is the preoptic area (POA). Nugent *et al*. found that steroid-induced gender identity in the POA is mediated by an epigenetic mechanism, specifically DNA methylation. The best characterized form of DNA methylation occurs at cytosine residues that are adjacent to guanines (CpG islands). DNA methyltransferase (DNMT) activity and CpG methylation were lower in the POA of male newborn rats than in those of female newborn rats. Subcutaneously injecting newborn female rats with estradiol induced a decrease in DNMT activity and CpG methylation for a few days. DNMT activity did not differ before or after the sensitive perinatal period, with or without estradiol injections. Intracerebral injection of the DNMT inhibitors zebularin or RG108 in female newborn rats mimicked the effect of estradiol on DNMT activity and produced neuronal morphology, protein marker patterns, and adult anxiety-related and sexual behavior more typical of male mice, without affecting the estrous cycle. In addition, adult female mice that lacked *Dnmt3a* in the POA from birth had anxiety and sexual behaviors more typical of male mice. However, unlike estradiol exposure, the masculinizing effects of knocking out Dnmt3a were not restricted to the sensitive perinatal period, suggesting that enduring DNA methylation maintains the female phenotype in the brain. RNA sequencing of the POA from postnatal male or female rats

revealed that a subset of genes derepressed by DNMT inhibition in females was associated with sexual differentiation. The findings further define how neurological gender identity is determined."

- The mechanisms underlying sexual differentiation of behavior and physiology in mammals and birds: relative contributions of sex steroids and sex chromosomes
    - "From a classical viewpoint, sex-specific behavior and physiological functions as well as the brain structures of mammals such as rats and mice, have been thought to be influenced by perinatal sex steroids secreted by the gonads. Sex steroids have also been thought to affect the differentiation of the sex-typical behavior of a few members of the avian order Galliformes, including the Japanese quail and chickens, during their development in ovo. However, recent mammalian studies that focused on the artificial shuffling or knockout of the sex-determining gene, Sry, have revealed that sex chromosomal effects may be associated with particular types of sex-linked differences such as aggression levels, social interaction, and autoimmune diseases, independently of sex steroid-mediated effects. In addition, studies on naturally occurring, rare phenomena such as gynandromorphic birds and experimentally constructed chimeras in which the composition of sex chromosomes in the brain differs from that in the other parts of the body, indicated that sex chromosomes play certain direct roles in the sex-specific differentiation of the gonads and the brain. In this article, we review the relative contributions of sex steroids and sex chromosomes in the determination of brain functions related to sexual behavior and reproductive physiology in mammals and birds."
- Early Prenatal Stress Epigenetically Programs Dysmasculinization in Second-Generation Offspring via the Paternal Lineage
    - "Studies have linked sex-biased neurodevelopmental disorders, including autism and schizophrenia, with fetal antecedents such as prenatal stress. Further, these outcomes can persist into subsequent generations, raising the possibility that aspects of heritability in these diseases involve epigenetic mechanisms. Utilizing a mouse model in which we previously identified a period in early gestation when stress results in dysmasculinized and stress-sensitive male offspring, we have examined programming effects in second-generation offspring of prenatally stressed (F2-S) or control (F2-C) sires. Examination of gene expression patterns during the perinatal sensitive period, when organizational gonadal hormones establish the sexually dimorphic brain, confirmed dysmasculinization in F2-S males, where genes important in neurodevelopment showed a female-like pattern. Analyses of the epigenomic miRNA environment detected significant reductions in miR-322, miR-574, and miR-873 in the F2-S male brain, levels that were again more similar to those of control females. Increased expression of a common gene target for these three miRNAs, β-glycan, was confirmed in these males. These developmental effects were associated with the transmission of a stress-sensitive phenotype and shortened anogenital distance in adult F2-S males. As confirmation that the miRNA environment is responsive to organizational testosterone, neonatal males administered the aromatase inhibitor formestane exhibited dramatic changes in brain miRNA patterns, suggesting that miRNAs may serve a previously unappreciated role in organizing the sexually dimorphic brain. Overall, these data support the existence of a sensitive period of early gestation when epigenetic programming of the male germline can occur, permitting transmission of specific phenotypes into subsequent generations."
- Sex differences in brain developing in the presence or absence of gonads

    - "Brain sexual differentiation results from the interaction of genetic and hormonal influences. This study used a unique agonadal mouse model to determine relative contributions of genetic and gonadal hormone influences in the differentiation of selected brain regions. SF-1 knockout (SF-1 KO) mice are born without gonads and adrenal glands and are not exposed to endogenous sex steroids during fetal/neonatal development. Consequently, male and female SF-1 KO mice are born with female external genitalia and if left on their own, die shortly after birth due to adrenal insufficiency. In this study, SF-1 KO mice were rescued by neonatal adrenal transplantation to examine their brain morphology in adult life. To determine potential brain loci that might mediate functional sex differences, we examined the area and distribution of immunoreactive calbindin and neuronal nitric oxide synthase in the preoptic area (POA) and ventromedial nucleus of the hypothalamus, two areas previously reported to be sexually dimorphic in the mammalian brain. A sex difference in the positioning of cells containing immunoreactive calbindin in a group within the POA was clearly gonad dependent based on the elimination of the sex difference in SF-1 KO mice. Several other differences in the area of ventromedial hypothalamus and in POA were maintained in male and female SF-1 KO mice, suggesting gonad-independent genetic influences on sexually dimorphic brain development."
- The Role of Androgen Receptors in the Masculinization of Brain and Behavior: What we've learned from the Testicular Feminization Mutation
    - "Many studies demonstrate that exposure to testicular steroids such as testosterone early in life masculinizes the developing brain, leading to permanent changes in behavior. Traditionally, masculinization of the rodent brain is believed to depend on estrogen receptors (ERs) and not androgen receptors (ARs). According to the aromatization hypothesis, circulating testosterone from the testes is converted locally in the brain by aromatase to estrogens, which then activate ERs to masculinize the brain. However, an emerging body of evidence indicates that the aromatization hypothesis cannot fully account for sex differences in brain morphology and behavior, and that androgens acting on ARs also play a role. The testicular feminization mutation (Tfm) in rodents, which produces a nonfunctional AR protein, provides an excellent model to probe the role of ARs in the development of brain and behavior. Tfm rodent models indicate that ARs are normally involved in the masculinization of many sexually dimorphic brain regions and a variety of behaviors, including sexual behaviors, stress response and cognitive processing. We review the role of ARs in the development of the brain and behavior, with an emphasis on what has been learned from Tfm rodents as well as from related mutations in humans causing complete androgen insensitivity."
- The control of sexual differentiation of the reproductive system and brain
    - "This review summarizes current knowledge of the genetic and hormonal control of sexual differentiation of the reproductive system, brain and brain function. While the chromosomal regulation of sexual differentiation has been understood for over 60 years, the genes involved and their actions on the reproductive system and brain are still under investigation. In 1990, the predicted testicular determining factor was shown to be the SRY gene. However, this discovery has not been followed up by elucidation of the actions of SRY, which may either stimulate a cascade of downstream genes, or inhibit a suppressor gene. The number of other genes known to be involved in sexual differentiation is increasing and the way in which they may interact is discussed. The

hormonal control of sexual differentiation is well-established in rodents, in which prenatal androgens masculinize the reproductive tract and perinatal oestradiol (derived from testosterone) masculinizes the brain. In humans, genetic mutations have revealed that it is probably prenatal testosterone that masculinizes both the reproductive system and the brain. Sexual differentiation of brain structures and the way in which steroids induce this differentiation, is an active research area. The multiplicity of steroid actions, which may be specific to individual cell types, demonstrates how a single hormonal regulator, e.g. oestradiol, can exert different and even opposite actions at different sites. This complexity is enhanced by the involvement of neurotransmitters as mediators of steroid hormone actions. In view of current environmental concerns, a brief summary of the effects of endocrine disruptors on sexual differentiation is presented."

- Sex differences in brain and behavior: hormones versus genes
    - "Sex determination is the commitment of an organism toward male or female development. Traditionally, in mammals, sex determination is considered equivalent to gonadal determination. Since the presence or the absence of the testes ultimately determines the phenotype of the external genitalia, sex determination is typically seen as equivalent to testis determination. But what exactly does sex determine? The endpoint of sex determination is almost invariably seen as the reproductive structures, which represent the most obvious phenotypic difference between the sexes. One could argue that the most striking differences between males and females are not the anatomy of the genitals, but the size of the gametes-considerably larger in females than males. In fact, there could be many different endpoints to sex determination, leading to differences between the sexes: brain sexual differences, behavioral differences, and susceptibility to disease. The central dogma of sexual differentiation, stemming initially from the gonad-transfer experiments of Alfred Jost, is that sexual dimorphisms of all somatic tissues are dependent on the testicular secretion of the developing fetus. In this chapter, we will take the example of sex differences in brain and behavior as an endpoint of sex determination. We will argue that genetic factors play a role in sexually dimorphic traits such as the number of dopaminergic cells in the mesencephalon, aggression, and sexual orientation, independently from gonadal hormones."
- Direct regulation of adult brain function by the male-specific factor SRY
    - "The central dogma of mammalian brain sexual differentiation has contended that sex steroids of gonadal origin organize the neural circuits of the developing brain. Recent evidence has begun to challenge this idea and has suggested that, independent of the masculinizing effects of gonadal secretions, XY and XX brain cells have different patterns of gene expression that influence their differentiation and function. We have previously shown that specific differences in gene expression exist between male and female developing brains and that these differences precede the influences of gonadal hormones. Here we demonstrate that the Y chromosome-linked, male-determining gene Sry is specifically expressed in the substantia nigra of the adult male rodent in tyrosine hydroxylase-expressing neurons. Furthermore, using antisense oligodeoxynucleotides, we show that Sry downregulation in the substantia nigra causes a statistically significant decrease in tyrosine hydroxylase expression with no overall effect on neuronal numbers and that this decrease leads to motor deficits in male rats. Our studies suggest that Sry directly affects the biochemical properties of the dopaminergic neurons of the nigrostriatal system and the specific motor behaviors they control. These results

demonstrate a direct male-specific effect on the brain by a gene encoded only in the male genome, without any mediation by gonadal hormones."

- Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice
    - "Across human cultures and mammalian species, sex differences can be found in the expression of aggression and parental nurturing behaviors: males are typically more aggressive and less parental than females. These sex differences are primarily attributed to steroid hormone differences during development and/or adulthood, especially the higher levels of androgens experienced by males, which are caused ultimately by the presence of the testis-determining gene Sry on the Y chromosome. The potential for sex differences arising from the different complements of sex-linked genes in male and female cells has received little research attention. To directly test the hypothesis that social behaviors are influenced by differences in sex chromosome complement other than Sry, we used a transgenic mouse model in which gonadal sex and sex chromosome complement are uncoupled. We find that latency to exhibit aggression and one form of parental behavior, pup retrieval, can be influenced by both gonadal sex and sex chromosome complement. For both behaviors, females but not males with XX sex chromosomes differ from XY. We also measured vasopressin immunoreactivity in the lateral septum, which was higher in gonadal males than females, but also differed according to sex chromosome complement. These results imply that a gene(s) on the sex chromosomes (other than Sry) affects sex differences in brain and behavior. Identifying the specific X and/or Y genes involved will increase our understanding of normal and abnormal aggression and parental behavior, including behavioral abnormalities associated with mental illness."
- Tissue-specific expression and regulation of sexually dimorphic genes in mice
    - "We report a comprehensive analysis of gene expression differences between sexes in multiple somatic tissues of 334 mice derived from an intercross between inbred mouse strains C57BL/6J and C3H/HeJ. The analysis of a large number of individuals provided the power to detect relatively small differences in expression between sexes, and the use of an intercross allowed analysis of the genetic control of sexually dimorphic gene expression. Microarray analysis of 23,574 transcripts revealed that the extent of sexual dimorphism in gene expression was much greater than previously recognized. Thus, thousands of genes showed sexual dimorphism in liver, adipose, and muscle, and hundreds of genes were sexually dimorphic in brain. These genes exhibited highly tissue-specific patterns of expression and were enriched for distinct pathways represented in the Gene Ontology database. They also showed evidence of chromosomal enrichment, not only on the sex chromosomes, but also on several autosomes. Genetic analyses provided evidence of the global regulation of subsets of the sexually dimorphic genes, as the transcript levels of a large number of these genes were controlled by several expression quantitative trait loci (eQTL) hotspots that exhibited tissue-specific control. Moreover, many tissue-specific transcription factor binding sites were found to be enriched in the sexually dimorphic genes."
- Partial demasculinization of several brain regions in adult male (XY) rats with a dysfunctional androgen receptor gene
    - "The adult rat posterodorsal medial amygdala (MePD) is sexually dimorphic in regional volume and neuronal soma size, both of which are larger in males than in females. This

sexual dimorphism is entirely dependent on adult circulating levels of testicular androgens, and both androgen and estrogen treatment can masculinize MePD structure. We examined male rats that are rendered androgen-insensitive by the testicular feminization mutation (*tfm*) of the androgen receptor (AR) gene to determine how a dysfunctional AR affects this and other brain sexual dimorphisms. In adult wild-type rats, the MePD in males had a greater regional volume, rostrocaudal extent, and soma size than in females. In genetic males, defective ARs affected some but not all of these indices: MePD volume and soma size in *tfm* males were intermediate between those of wild-type males and females, but the rostrocaudal extent of the MePD was unaffected by the mutation, being as great in *tfm* males as in wild-type males. Regional volume and soma size in the suprachiasmatic nucleus was reduced in *tfm* males compared with wild-type males, suggesting that AR normally affects this region in male rats. Interestingly, whereas volume of the sexually dimorphic nucleus of the preoptic area was unaffected by the *tfm* allele, soma size in this region was reduced in *tfm* males compared with wild-type males. Although estrogen receptor activation has been shown to be vital for masculinization of the rodent brain, our results indicate that ARs also contribute to this process in several brain regions."

- Are XX and XY brain cells intrinsically different?
    - "In mammals and birds, the sex of the gonads is determined by genes on the sex chromosomes. For example, the mammalian Y-linked gene Sry causes testis differentiation. The testes then secrete testosterone, which acts on the brain (often after conversion to estradiol) to cause masculine patterns of development. If this were the only reason for sex differences in neural development, then XX and XY brain cells would have to be deemed otherwise equivalent. This equivalence is doubtful because of recent experimental results demonstrating that some XX and XY tissues, including the brain, are sexually dimorphic even when they develop in a similar endocrine environment. Although X and Y genes probably influence brain phenotype in a sex-specific manner, much more information is needed to identify the magnitude and character of these effects."
- Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation
    - "The classic view of brain sexual differentiation and behavior is that gonadal steroid hormones act directly to promote sex differences in neural and behavioral development. In particular, the actions of testosterone and its metabolites induce a masculine pattern of brain development, while inhibiting feminine neural and behavioral patterns of differentiation. However, recent evidence indicates that gonadal hormones may not solely be responsible for sex differences in brain development and behavior between males and females. Here we examine an alternative hypothesis that genes, by directly inducing sexually dimorphic patterns of neural development, can influence the sexual differences between male and female brains. Using microarrays and RT-PCR, we have detected over 50 candidate genes for differential sex expression, and confirmed at least seven murine genes which show differential expression between the developing brains of male and female mice at stage 10.5 days post coitum (dpc), before any gonadal hormone influence. The identification of genes differentially expressed between male and female brains prior to gonadal formation suggests that genetic factors may have roles in influencing brain sexual differentiation."
- A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits

    - "We tested the hypothesis that genes encoded on the sex chromosomes play a direct role in sexual differentiation of brain and behavior. We used mice in which the testis-determining gene (Sry) was moved from the Y chromosome to an autosome (by deletion of Sry from the Y and subsequent insertion of an Sry transgene onto an autosome), so that the determination of testis development occurred independently of the complement of X or Y chromosomes. We compared XX and XY mice with ovaries (females) and XX and XY mice with testes (males). These comparisons allowed us to assess the effect of sex chromosome complement (XX vs XY) independent of gonadal status (testes vs ovaries) on sexually dimorphic neural and behavioral phenotypes. The phenotypes included measures of male copulatory behavior, social exploration behavior, and sexually dimorphic neuroanatomical structures in the septum, hypothalamus, and lumbar spinal cord. Most of the sexually dimorphic phenotypes correlated with the presence of ovaries or testes and therefore reflect the hormonal output of the gonads. We found, however, that both male and female mice with XY sex chromosomes were more masculine than XX mice in the density of vasopressin-immunoreactive fibers in the lateral septum. Moreover, two male groups differing only in the form of their Sry gene showed differences in behavior. The results show that sex chromosome genes contribute directly to the development of a sex difference in the brain."
- Sex chromosome genes directly affect brain sexual differentiation
    - "Sex differences in the brain are caused by differences in gonadal secretions: higher levels of testosterone during fetal and neonatal life cause the male brain to develop differently than the female brain. In contrast, genes encoded on the sex chromosomes are not thought to contribute directly to sex differences in brain development, even though male (XY) cells express Y-chromosome genes that are not present in female (XX) cells, and XX cells may have a higher dose of some X-chromosome genes. Using mice in which the genetic sex of the brain (XX versus XY) was independent of gonadal phenotype (testes versus ovaries), we found that XY and XX brain cells differed in phenotype, indicating that a brain cell's complement of sex chromosomes may contribute to its sexual differentiation."
- Genetically triggered sexual differentiation of brain and behavior
    - "The dominant theory of sexual differentiation of the brain holds that sex differences in brain anatomy and function arise because of the action of gonadal steroids during embryonic and neonatal life. In mammals, testicular steroids trigger masculine patterns of neural development, and feminine patterns of neural development occur in the absence of such testicular secretions. In contrast, gonadal differentiation in mammals is not initiated by hormonal mechanisms, but is regulated by the action of gene products such as SRY, a testis-determining gene on the Y chromosome. This paper argues that such genetic, nonhormonal signals may also trigger specific examples of sexual differentiation of the brain. This thesis is supported by two arguments. The first is that "direct genetic" (i.e., nonhormonal) control of sexual differentiation may be as likely to evolve as hormonal control. The second line of argument is that neural and nonneural dimorphisms have already been described that are not well explained by classical theories of steroid-dependent sexual differentiation and for which other factors need to be invoked."
- Review A genetic approach to dissect sexually dimorphic behaviors

- "It has been known since antiquity that gender-specific behaviors are regulated by the gonads. We now know that testosterone is required for the appropriate display of male patterns of behavior. Estrogen and progesterone, on the other hand, are essential for female typical responses. Research from several groups also indicates that estrogen signaling is required for male typical behaviors. This finding raises the issue of the relative contribution of these two hormonal systems in the control of male typical behavioral displays. In this review we discuss the findings that led to these conclusions and suggest various genetic strategies that may be required to understand the relative roles of testosterone and estrogen signaling in the control of gender specific behavior."

Miscellaneous Studies and Articles

Miscellaneous Studies

- Discordant Sexual Identity in Some Genetic Males with Cloacal Exstrophy Assigned to Female Sex at Birth
  - "Eight of the 14 subjects assigned to female sex declared themselves male during the course of this study, whereas the 2 raised as males remained male. Subjects could be grouped according to their stated sexual identity. Five subjects were living as females; three were living with unclear sexual identity, although two of the three had declared themselves male; and eight were living as males, six of whom had reassigned themselves to male sex. All 16 subjects had moderate-to-marked interests and attitudes that were considered typical of males. Follow-up ranged from 34 to 98 months."
- AMH and AMH receptor defects in persistent Müllerian duct syndrome
  - "Anti-Müllerian hormone (AMH) produced by fetal Sertoli cells is responsible for regression of Müllerian ducts, the anlage for uterus and Fallopian tubes, during male sex differentiation. A member of the transforming growth factor-beta superfamily, AMH signals through two transmembrane receptors, type II which is specific and type I receptors, shared with the bone morphogenetic protein family. Mutations of the AMH and AMH receptor type II (AMHR-II) genes lead to persistence of the uterus and Fallopian tubes in males. Both conditions are transmitted according to a recessive autosomal pattern and are symptomatic only in males. Affected individuals are otherwise normally virilized, undergo normal male puberty; and may be fertile if testes, tightly attached to the Fallopian tubes, can be replaced in the scrotum. Approximately 85% of the cases are due, in similar proportions, to mutations of the AMH or AMHR-II gene. The genetic background does not influence the phenotype, the only difference is the level of circulating AMH which is normal for age in AMHR-II mutants and usually low or undetectable in AMH gene defects. This is due to lack of secretion, explained by the localization of the mutations in critical regions, based on the assumed 3D structure of the molecule. Similarly, lack of translocation to the surface membrane is responsible for the inactivity of AMHR-II molecules bearing mutations in the extracellular domain. In 15% of cases, the cause of the persistent Mullerian duct syndrome is unknown and could be related to complex malformations of the urogenital region, unrelated to AMH physiology."
- True hermaphroditism with oogenesis and spermatogenesis

    - No abstract available.
- Somatic Sex Reprogramming of Adult Ovaries to Testes by FOXL2 Ablation
    - "In mammals, the transcription factor SRY, encoded by the Y chromosome, is normally responsible for triggering the indifferent gonads to develop as testes rather than ovaries. However, testis differentiation can occur in its absence. Here we demonstrate in the mouse that a single factor, the forkhead transcriptional regulator FOXL2, is required to prevent transdifferentiation of an adult ovary to a testis. Inducible deletion of *Foxl2* in adult ovarian follicles leads to immediate upregulation of testis-specific genes including the critical SRY target gene *Sox9*. Concordantly, reprogramming of granulosa and theca cell lineages into Sertoli-like and Leydig-like cell lineages occurs with testosterone levels comparable to those of normal XY male littermates. Our results show that maintenance of the ovarian phenotype is an active process throughout life. They might also have important medical implications for the understanding and treatment of some disorders of sexual development in children and premature menopause in women."
- Dichotic Listening, Handedness, Brain Organization and Transsexuality
    - "This study investigated the functional brain organization of 68 male-to-female (MtF) transwomen and 26 female-to-male (FtM) transmen by comparing their performance with 36 typical male and 28 typical female controls on two indicators of cerebral lateralization: dichotic listening and handedness. A sex-differentiating dichotic test and a handedness questionnaire were administered. It was hypothesized that the MtF participants' dichotic performance would be significantly different from the control males and resemble the control female pattern. This hypothesis was supported. It was also hypothesized that the FtM dichotic pattern would be significantly different from the control females and would resemble the control male pattern. This hypothesis was not supported. Finally, it was hypothesized that there would be significantly more nonexclusive right-handers in both trans-groups. This hypothesis was supported. Taken together, the dichotic and handedness data reported here indicate that the MtF and FtM conditions are not mirror images in terms of the verbal-auditory aspects of their brain organization and neurobiology plays an important role, particularly in the development of the male-to-female trans-condition."
- Disordered or Just Different Others
    - Disordered Or Just Different? Myth, Science, and Sexuality
        - "Learn why the biblical binary sexual system is unnatural! A physiologist's perspective on core sexuality: sexual identity, sexual orientation, sex versus gender, anthropological observations. "Disordered or Just Different" culminates with a scientific indictment of the (pseudo)medical practitioners who are too quick to classify the merely different as disordered and who continue to harm gay, lesbian, transsexual, and intersex peoples."
    - Link 2
        - Large collection of slideshows by Veronica Drantz, PHD
- Digit ratio (2D:4D) is associated with traffic violations for male frequent car drivers
    - "Digit ratio (2D:4D) is a putative marker of prenatal hormone exposure. A lower digit ratio has been suggested as an index of higher testosterone relative to estrogen exposure during prenatal development. Digit ratio has been associated with a variety of psychological sex-dimorphic variables, including spatial orientation, aggression, or risk-taking behavior. The present study aimed to relate digit ratio to traffic violations for a

male sample (N = 77) of frequent car drivers. Digit ratio was assessed via printout scans of the hand, and traffic offense behavior was assessed via self-reported penalty points as registered by the Central Register of Traffic Offenders in Germany. In addition, social desirability and sensation seeking were recorded. Results showed that digit ratio was inversely related to penalty point entries, suggesting more traffic violations for individuals with higher prenatal testosterone exposure. Sensation seeking was positively associated with traffic violations, but there was no relationship between sensation seeking and digit ratio, proposing additive effects of both variables. The results suggest that prenatal androgen exposure might be related to traffic violations for frequent car drivers."

- Finger length ratio (2D:4D) in adults with gender identity disorder
    - "From early childhood, gender identity and the 2nd to 4th finger length ratio (2D:4D) are discriminative characteristics between sexes. Both the human brain and 2D:4D may be influenced by prenatal testosterone levels. This calls for an examination of 2D:4D in patients with gender identity disorder (GID) to study the possible influence of prenatal testosterone on gender identity. Until now, the only study carried out on this issue suggests lower prenatal testosterone levels in right-handed male-to-female GID patients (MtF). We compared 2D:4D of 56 GID patients (39 MtF; 17 female-to-male GID patients, FtM) with data from a control sample of 176 men and 190 women. Bivariate group comparisons showed that right hand 2D:4D in MtF was significantly higher (feminized) than in male controls, but similar to female controls. The comparison of 2D:4D ratios of biological women revealed significantly higher (feminized) values for right hands of right handed FtM. Analysis of variance confirmed significant effects for sex and for gender identity on 2D:4D ratios but not for sexual orientation or for the interaction among variables. Our results indirectly point to the possibility of a weak influence of reduced prenatal testosterone as an etiological factor in the multifactorially influenced development of MtF GID. The development of FtM GID seems even more unlikely to be notably influenced by prenatal testosterone."
- 2D:4D finger-length ratios in children and adults with gender identity disorder
    - "Previous research suggests that prenatal testosterone affects the 2D:4D finger ratio in humans, and it has been speculated that prenatal testosterone also affects gender identity differentiation. If both things are true, then one would expect to find an association between the 2D:4D ratio and gender identity. We measured 2D:4D in two samples of patients with gender identity disorder (GID). In Study 1, we compared the 2D:4D ratios of 96 adult male and 51 female patients with GID to that of 90 heterosexual male and 112 heterosexual female controls. In Study 2, we compared the 2D:4D ratios of 67 boys and 34 girls with GID to that of 74 control boys and 72 control girls. In the sample of adults with GID, we classified their sexual orientation as either homosexual or non-homosexual (in relation to their birth sex) to examine whether or not there were any within-group differences as a function of sexual orientation. In the sample of adult men with GID (both homosexual and non-homosexual) and children with GID, we found no evidence of an altered 2D:4D ratio relative to same-sex controls. However, women with GID had a significantly more masculinized ratio compared to the control women. This last finding was consistent with the prediction that a variance in prenatal hormone exposure contributes to a departure from a sex-typical gender identity in women."
- On the relation between 2D:4D and sex-dimorphic personality traits

    - "Several personality traits, including aggressiveness and sensation seeking, have been hypothesized to be influenced by prenatal androgen exposure, though evidence for this proposition is limited. We investigated whether individual differences in aggressiveness, sensation seeking, and several prosocial personality traits can be predicted from differences in the 2D:4D digit ratio, a putative marker of prenatal androgen activity. A total of 164 undergraduates (87 men, 77 women) completed self-report measures of physical and verbal aggression, as well as a standardized measure of sensation seeking, and five scales to assess empathy, nurturance, expressivity/femininity, instrumentality/masculinity, and assertiveness. Two sex-dimorphic tests of spatial ability also were included. Men had a lower 2D:4D ratio than women, confirming the typical sex difference in digit proportions. Significant sex differences were observed on 10 of the 11 personality scales purported to show sex differences and on both tests of spatial ability. The 2D:4D ratio was a significant predictor of scores on three of the four aggression subscales, total aggression, thrill and adventure seeking, and total sensation-seeking, in the sample as a whole and in women. In men, correlations with 2D:4D were significant only for total sensation-seeking and verbal aggression. In both sexes, lower 2D:4D ratios were associated with increased aggressiveness and sensation seeking. For the spatial tests, there was no evidence of any association with 2D:4D in either men or women. The 2D:4D digit ratio may be a valid, though weak, predictor of selective sex-dependent traits that are sensitive to testosterone."
- Typical female 2nd–4th finger length (2D:4D) ratios in male-to-female transsexuals—possible implications for prenatal androgen exposure
    - "Prenatal exposure to androgens has been implicated in transsexualism but the etiology of the condition remains unclear. The ratio of the 2nd to the 4th (2D:4D) digit lengths has been suggested to be negatively correlated to prenatal androgen exposure. We wanted to assess differences in 2D:4D ratio between transsexuals and controls.
      Sixty-three male-to-female transsexuals (MFT), 43 female-to-male transsexuals (FMT), and 65 female and 58 male controls were included in the study. Photocopies of the palms and digits of the hands were taken of all subjects and 2D:4D ratios were measured, according to standard published procedures.
      Comparison between right-handed individuals revealed that the right-hand 2D:4D in MFT is higher than in control males but similar to that observed in control females. In FMT we found no differences in 2D:4D relative to control females. Our findings support a biological etiology of male-to-female transsexualism, implicating decreased prenatal androgen exposure in MFT. We have found no indication of a role of prenatal hormone exposure in female-to-male transsexualism."
- Finger Length Ratios in Serbian Transsexuals
    - "Atypical prenatal hormone exposure could be a factor in the development of transsexualism. There is evidence that the 2nd and 4th digit ratio (2D : 4D) associates negatively with prenatal testosterone and positively with estrogens. The aim was to assess the difference in 2D : 4D between female to male transsexuals (FMT) and male to female transsexuals (MFT) and controls. We examined 42 MFT, 38 FMT, and 45 control males and 48 control females. Precise measurements were made by X-rays at the ventral surface of both hands from the basal crease of the digit to the tip using vernier calliper. Control male and female patients had larger 2D : 4D of the right hand when compared to the left hand. Control male's left hand ratio was lower than in control female's left hand.

There was no difference in 2D : 4D between MFT and control males. MFT showed similar 2D : 4D of the right hand with control women indicating possible influencing factor in embryogenesis and consequently finger length changes. FMT showed the lowest 2D : 4D of the left hand when compared to the control males and females. Results of our study go in favour of the biological aetiology of transsexualism."

- Empathizing, systemizing and finger length ratio in a Swedish sample
    - "The Empathy- and Systemizing Quotients (EQ and SQ, respectively; Baron-Cohen, 2003) were determined in a Swedish sample consisting mainly of university undergraduates. Females had significantly higher EQ than males, who in turn scored higher on the SQ inventory. Gender explained 12-14% of the variation. Males were strikingly overrepresented in the group defined by a high SQ/low EQ profile or by a large SQ - EQ difference; females dominated among people with a low SQ/high EQ profile or by a large EQ - SQ difference. Students majoring in the natural sciences had higher SQs than psychology majors, but in both groups the gender difference in SQ and EQ was strong. For each participant a weighted composite score was generated by multivariate processing of the EQ and SQ data (Partial Least Square Discriminant Analysis). These scores were associated in a sex-linked fashion to a biometric measure reflecting prenatal testosterone exposure, i.e. the ratio between index (2D)- and ring (4D) finger lengths. In males a high (female-typical) 2D:4D ratio predicted an enhanced tendency to empathize and a reduced tendency to systemize; in women, by contrast, the 2D:4D ratio was unrelated to these traits. The present research confirms earlier work of a gender difference in EQ and SQ. The difference appears robust as it appears as large in Sweden (a country with high cultural gender-equality) as in countries with considerably lower gender-equality."
- Investigating digit ratio (2D:4D) in a highly male-dominated occupation: the case of firefighters
    - "Second-to-fourth digit ratio (2D:4D), a widely studied putative marker for masculinization through prenatal androgen exposure, is lower (more masculinized) in athletes than in general population controls, and athletes with lower 2D:4D have higher sporting success. Occupations differ markedly in perceived masculinity and actual maleness (sex ratios), but these givens have not yet been picked up and utilized in 2D:4D research. Accordingly, this study extended existing accounts on 2D:4D in athletes to a novel approach: 2D:4D and possible relationships to a variety of candidate variables (demographic, fertility-related, psychological, and other) were investigated in firefighters, a highly male-dominated occupation. Contrary to expectation, 2D:4D in firefighters (N = 134) was not lower than in local male population controls. Lower 2D:4D corresponded to lower service ranks. Replicating previous findings either unequivocally or partly, lower 2D:4D was associated with larger family size, later sibling position, left-handedness, and higher scores in the disinhibition component of sensation seeking. Not replicating prior evidence, 2D:4D was unrelated to body-mass index, offspring sex ratio, and sporting performance level. Novel findings included low 2D:4D in those with low relationship satisfaction and in cigarette smokers, especially among heavy smokers. Absolute finger length, a positive correlate of pubertal-adolescent androgen levels, was also considered. This marker showed negative associations with relationship consensus and satisfaction and positive ones with perceived quality of relationship alternatives and the experience seeking component of sensation seeking. The merits of this additional marker, relative to

2D:4D, for supplementing studies of possible sex-hormonal effects on personality and directions for future inquiry along these lines are discussed."

- Evidence for assortative mating on digit ratio (2D:4D), a biomarker for prenatal androgen exposure
    - "The second-to-fourth digit ratio (2D:4D) presents an anatomical sex difference in humans. On average, men tend to have lower 2D:4D compared with women. There is fairly strong evidence for a role of the 2D:4D ratio as a biomarker for the organizational (permanent) effects of prenatal testosterone on the brain and behaviour. Recently, an accumulating research programme has shown 2D:4D to be related to a multitude of sex-dependent, hormonally influenced biosocial traits and phenotypes which reach into the domains of ability, behaviour, fertility, health, personality and sexuality. This study investigated the degree of assortative mating (spousal similarity) in a sample of 239 native Austrian couples of parental or grandparental age, all of them having reproduced. Results included: (i) significant spousal correlations of +0.19 and +0.18 for right-hand and left-hand 2D:4D, respectively, and +0.24 for average 2D:4D; (ii) no assortative mating effect on the right-minus-left difference in 2D:4D; (iii) indications consistent with a possible generational decrease of spousal similarity in 2D:4D; (iv) a prevalence of couples with a lower right-hand 2D:4D observed in the husband compared with his wife; and (v) relations of spousal 2D:4D patterns to spousal age differences, such that matings of men with more male-typical trait expressions (namely, a generally low right-hand 2D:4D or showing a lower right-minus-left 2D:4D difference than their wives) implicated larger male-minus-female age differences, i.e. younger wives. It is argued that assortative mating on 2D:4D operates indirectly and may be mediated through the assortment on other, more perceptible, physical traits and psychological phenotypes that entertain associations with 2D:4D and are relevant for courtship and mate choice."
- Minireview: Organizational hypothesis: instances of the fingerpost
    - "There is now compelling evidence that the ratio of the length of the second digit divided by the length of the fourth digit (2D:4D) is affected by prenatal androgens in humans. This ratio is greater in females than males from fetal life through adulthood, correlates with polymorphism in the androgen receptor gene in men, is feminine in XY androgen insensitivity syndrome, and masculinized in congenital adrenal hyperplasia. Using 2D:4D as a correlate, researchers have found evidence that prenatal androgens affect many sexually differentiated human behaviors, including sexual orientation in women (but not in men), attention deficit disorder, autism, eating disorders, aggression, and risk-taking. In each case, lower 2D:4D, indicative of greater prenatal androgen stimulation, is associated with behavior more commonly displayed by males than females. The correlation between 2D:4D and prenatal androgen stimulation is too imperfect to accurately predict the phenotype of a particular individual, even in terms of sex. However, digit ratio is the best available retrospective marker of average differences in prenatal androgen stimulation between groups of people, and/or correlations of prenatal androgen stimulation with particular behaviors and characteristics within a group. Thus digit ratios offer a valid test of the organizational hypothesis that androgens act early in life to masculinize various human behaviors."
- Activating effects of cross-sex hormones on cognitive functioning: a study of short-term and long-term hormone effects in transsexuals

    - "In an earlier study we demonstrated that 3 months of cross-sex hormone treatment clearly influenced cognitive functioning in transsexuals. The aims of the present study were to examine: (a) whether we could replicate these findings in a new group of transsexuals; (b) whether a similar pattern of change could be found for novel tasks, i.e. tasks, not used in the previous study, that measured closely related cognitive abilities; (c) whether the cognitive changes following cross-sex hormone treatment had stabilized after 3 months or continued to develop over a period of 1 year; and finally, (d) whether the effects were quickly reversible when the hormone treatment was temporarily stopped. Again a pronounced effect of androgen treatment was found on spatial ability in female-to-male transsexuals (FMs) over a period of one and a half years. As expected, untreated male-to-female transsexuals (MFs) had higher scores on visuo-spatial tasks than untreated FMs; after 3 months of cross-sex hormone treatment, the group difference had disappeared, while after about 10 months of hormone treatment, the sex difference was reversed. These effects did not disappear after termination of cross-sex hormone therapy for a period of 5 weeks, but continued to change slightly in the same direction. Earlier findings of an opposite effect of cross-sex hormones on verbal fluency (i.e. MFs improved and FMs deteriorated after 3 months of cross-sex hormone treatment) were not replicated in this study, nor did we find an hormonal influence on other cognitive functions. This study shows that testosterone had an enhancing, and not quickly reversible effect, on spatial ability performance, but no deteriorating effect on verbal fluency in adult women (FMs). In contrast, anti-androgen treatment in combination with estrogen therapy had no declining effect on spatial ability, nor an enhancing effect on verbal fluency in adult men (MFs)."
- The dermatoglyphic characteristics of transsexuals: is there evidence for an organizing effect of sex hormones
    - "It has been proposed that gender identity and sexual orientation are influenced by the prenatal sex steroid milieu. Human dermatoglyphics and brain asymmetry have also been ascribed to prenatal hormone levels. This study investigated dermatoglyphics (total ridge count and finger ridge asymmetry) in 184 male-to-female transsexuals and 110 female-to-male transsexuals. In a subgroup, the relationship between dermatoglyphic asymmetry and spatial ability was tested. All investigations included controls. For all subjects hand preference and sexual orientation were noted. We hypothesized that the dermatoglyphics of male-to-female transsexuals would show similarities with control women and those of female-to-male transsexuals with control men. Our results showed a trend for a sex difference in total ridge count ($P<.1$) between genetic males and females, but no difference in directional asymmetry was found. Contrary to our expectations, the total ridge count and finger ridge asymmetry of transsexuals were similar to their genetic sex controls. Additionally, directional asymmetry was neither related to sexual orientation, nor to different aspects of spatial ability. In conclusion, we were unable to demonstrate that our chosen dermatoglyphic variables, total ridge count and finger ridge asymmetry are related to gender identity and sexual orientation in adult transsexuals. Hence, we found no support for a prenatal hormonal influence on these characteristics, at least insofar as dermatoglyphics may be regarded as a biological marker of organizing hormonal effects."
- Sexual functioning in transsexuals following hormone therapy and genital surgery: a review

- "*Results:* Contrary to early views, transsexualism does not appear to be associated with a hyposexual condition. In MtF transsexuals, rates of hypoactive sexual desire disorder (HSDD) are similar to those found in the general female population. In FtM transsexuals, sexual desire appears unequivocally to increase following SRS. Studies with MtF transsexuals have revealed not only vasocongestion, but also the secretion of fluid during sexual arousal. Research on sexual arousal in FtM transsexuals is sorely lacking, but at least one study indicates increased arousal following SRS. The most substantial literature on sexual functioning in postoperative transsexuals pertains to orgasm, with most reports indicating moderate to high rates of orgasmic functioning in both MtF and FtM transsexuals.
  Conclusions: Based on the available literature, transsexuals appear to have adequate sexual functioning and/or high rates of sexual satisfaction following SRS. Further research is required to understand fully the effects of varying types and dosages of cross-sex hormone therapies and particular SRS techniques on sexual functioning."

- Hormone-related tumors in transsexuals receiving treatment with cross-sex hormones
  - "*Results:* In male-to-female transsexuals receiving estrogen administration, lactotroph adenomas, breast cancer, and prostate cancer have been reported. In female-to-male transsexuals receiving treatment with testosterone, a single case of breast carcinoma and several cases of ovarian cancer have been reported. So far endometrial cancer has not been encountered though it remains a potential malignant development.
    Conclusions: There are so far only a few cases of hormone-related cancer in transsexuals. There may be an underreporting. The probability of a hormone-related tumor increases with the duration of exposure to cross-sex hormones and the aging of the population of transsexuals."
- Spatial ability and prenatal androgens: meta-analyses of congenital adrenal hyperplasia and digit ratio (2D:4D) studies
  - "Hormonal manipulations indicate that early androgens organize sex differences in spatial ability in laboratory rats. In humans, spatial ability is also sexually dimorphic, and information about the effects of prenatal androgens on spatial ability can be obtained from studies of congenital adrenal hyperplasia (CAH) and the ratio of the second and fourth finger lengths (2D:4D). CAH is characterized by prenatal overproduction of adrenal androgens and several lines of evidence suggest that 2D:4D reflects prenatal androgen exposure. Some studies have found that these proxy measures of prenatal androgens predict spatial ability, others have found no significant relationship, and yet others have obtained results in the opposite direction. In light of these mixed findings, we conducted meta-analyses of published literature and unpublished results to determine if, across studies, either of these indicators of prenatal androgens predicts performance on spatial tasks that show a male advantage. In addition, we applied a trim and fill analysis to the data in search of asymmetry that might be an indication of publication bias. Results indicated that females with CAH perform better on these spatial tasks, and CAH males perform worse, than do controls. Little or no relationship exists between 2D:4D and spatial ability. Implications for possible hormonal contributions and the developmental timing of sex differences in spatial cognition are discussed."

Familial Studies

- Pacific Center for Sex and Society - Identical Reared Apart Twins Concordant for Transsexuality
    - "A growing twin-based literature supports genetic influence on gender identity development.[1] An international survey of adult transsexual twin pairs reported transition concordance values of 33.3% (13/39) for identical [monozygotic (MZ)] male pairs and 22.9% (8/35) for MZ female pairs. By contrast, transition concordance values for fraternal [dizygotic (DZ)] male and female twins were zero or approached zero (1/36), consistent with genetic influence.[2] Here, we report the first case of transsexualism in both reared apart brothers of a male-to-female MZ twin pair."
- Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls.
    - "In animals it has been shown that exposure to sex hormones is influenced by intrauterine position. Thus fetuses located between two male fetuses are exposed to higher levels of testosterone (T) than fetuses situated between two female fetuses or one female and one male fetus. In a group of opposite-sex (OS) twin girls and same-sex (SS) twin girls a potential effect of prenatal exposure to testosterone (T) on functional cerebral lateralization was investigated. We hypothesized that prenatal exposure to T would result in a more masculine, i.e. a more lateralized pattern of cerebral lateralization in OS twin girls than in SS twin girls. An auditory-verbal dichotic listening task (DLT) was used as an indirect method to study hemispheric specialization. Firstly, we established a sex difference on the DLT. Compared with SS girls, OS twin boys showed a more lateralized pattern of processing verbal stimuli. Secondly, as predicted OS girls had a more masculine pattern of cerebral lateralization, than SS girls. These findings support the notion of an influence of prenatal T on early brain organization in girls."
- Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls
    - "Men and women differ in temperament and personality traits, such as aggression and sensation seeking. The sex hormone testosterone could play a role in the origin of these differences, but it remains unclear how and when testosterone could have these effects. One way to investigate the prenatal exposure effect of testosterone is to compare opposite-sex (OS) and same-sex (SS) female twins. It has been suggested that OS twin girls are exposed prenatally to elevated testosterone levels and that this may result in some masculinization of their personality and behavior. We measured sexually dimorphic traits and circulating testosterone levels in 13-year-old OS (n = 74) and SS (n = 55) twins. Testosterone levels showed a clear circadian rhythm, with higher levels in the morning than in the afternoon. Testosterone was higher in boys than girls, but similar in OS and SS twin girls. Testosterone was not in any way systematically related to the different personality traits. However, a sex difference in aggression proneness was observed, and OS girls showed a more masculine pattern of aggression proneness than the SS girls. It is argued that it is unlikely that this difference is due to social factors, such as a gender-specific upbringing."
- Evaluating the twin testosterone transfer hypothesis: a review of the empirical evidence
    - "In this paper we review the evidence that fetuses gestated with a male co-twin are masculinized in development, perhaps due to the influence of prenatal androgens: the so-called twin testosterone transfer (TTT) hypothesis. Evidence from studies of behavioral, perceptual, cognitive, morphological and physiological traits in same- and

opposite-sex human twins is considered. Apart from two studies reporting increases in aspects of sensation-seeking for females with a male rather than a female co-twin, there is sparse evidence supporting the TTT hypothesis in behavioral studies. Outcomes from studies of perception (in particular otoacoustic emissions) and cognition (in particular vocabulary acquisition and visuo-spatial ability) provide more consistent evidence in support of masculinized performance in twins with a male co-twin compared to twins with a female co-twin. The outcomes favorable to the TTT hypothesis for otoacoustic emissions and visuo-spatial ability are restricted to females. Studies of physiology and morphology (e.g., brain volume, tooth size and 2D:4D ratio) also show some influence of co-twin sex, but again these effects are often restricted to female twins. Because females produce little endogenous testosterone, the effects of gestation with a male co-twin may be more pronounced in females than males. Thus, while uneven, the evidence for the TTT hypothesis is sufficient to warrant further investigation, ideally using large samples of same- and opposite-sex twins, along with control groups of same- and opposite-sex siblings when the characteristics assessed are potentially open to social influences."

- "Any decision is better than none" decision-making about sex of rearing for siblings with 17beta-hydroxysteroid-dehydrogenase-3 deficiency
    - "Children with 17beta-hydroxysteroid-dehydrogenase-3 (17beta-HSD-3) deficiency have a defect of testosterone biosynthesis with subsequent diminished virilization in XY individuals. Some are raised as girls and some as boys. There were two purposes of this case report: First, it analyzed the process of decision-making in a family with a pair of siblings with identical mutations leading to 17beta-HSD-3 deficiency whose parents chose to raise one child as a boy and one as a girl. This analysis was based on narrative interviews with the parents. Second, we assessed the gender role behavior and gender identity in the children to examine if the psychosexual development of these children correspond with the sex of rearing their parents chose. When participating in the study, the children were 7 (boy) and 5 (girl) years old. Parents described a difficult process of decision-making and voiced concerns about lack of appropriate and understandable information, and anticipated decision regret. However, they did not feel that the decision to "normalize" the external genitalia should have been deferred. Both children appeared to show age-typical gender-related behavior and did not show any signs of physical or mental distress."

Articles and Reviews

- Potential Therapeutic Errors When Using Binary Based Terminology to Explain the Gender Variant Condition
    - "Five of the most common terms used when working with people struggling with gender issues are "**gender dysphoria**," "**Gender Identity Disorder**," "**female-to-male**," "**male-to-female**," and "**sex change**." Because these terms are based on a male/female binary norm, they may not be apt descriptors of people who are gender variant. Or worse, if taken literally they could lead to practitioners misdiagnosing the condition, clients misinterpreting their situation and both client and practitioner having unrealistic expectations for treatment outcome. "

- American Transman on "Male Gender Identity in an individual with Complete Androgen Insensitivity Syndrome"
    - Part 1
    - Part 2
    - Part 3
    - Part 4
- Biological and Psychosocial Correlates of Adult Gender-Variant Identities: a Review
    - "This article reviews research on biological and psychosocial factors relevant to the etiology of gender-variant identities. There is evidence for a genetic component of gender-variant identities through studies of twins and other within-family concordance and through studies of specific genes. Evidence that prenatal androgens play a role comes from studies that have examined finger length ratios (2D:4D), prevalence of polycystic ovary syndrome among female-to-male transsexuals, and individuals with intersex and related conditions who are more likely to have reassigned genders. There is also evidence that transsexuals have parts of their brain structure that is typical of the opposite birth-assigned gender. A greater likelihood of non-right-handedness suggests developmental instability may also contribute as a biological factor. There is a greater tendency for persons with gender-variant identities to report childhood abuse and a poor or absent relationship with parents. It is unclear if this is a cause or effect of a gender-variant identity. Parental encouragement of gender-variance is more common among individuals who later develop a gender-variant identity. We conclude that biological factors, especially prenatal androgen levels, play a role in the development of a gender-variant identity and it is likely that psychosocial variables play a role in interaction with these factors."
- Atypical Gender Development: a review
    - "In sum, gender identity, whether consistent or inconsistent with other sex characteristics, may be understood to be "much less a matter of choice and much more a matter of biology" (Coolidge *et al.*, 2000). The scientific evidence supports the paradigm that transsexualism is strongly associated with the neurodevelopment of the brain (Zhou *et al.*, 1995; Kruijver *et al.*, 2000). It is clear that the condition cannot necessarily be overcome by "consistent psychological socialisation as male or female from very early childhood" and it is not responsive to psychological or psychiatric treatments alone (Green, 1999). It is understood that during the fetal period the brain is potentially subject to the organising properties of sex hormones (Kruijver *et al.*, 2000; 2001; 2002; 2003). In the case of transsexualism, these effects appear to be atypical, resulting in sex-reversal in the structure of the BSTc, and possibly other, as yet unidentified, loci (Kruijver, 2004). The etiological pathways leading to this inconsistent development almost certainly vary from individual to individual, so no single route is likely to be identified. Different genetic, hormonal and environmental factors, acting separately or in combination with each other, are likely to be involved in influencing the development of the psychological identification as male or female. Psychosocial factors and cultural mores are likely to impact on outcomes (Connolly, 2003)."
- (PDF) The Transsexual Phenomenon: A Counter-History
    - "Within a relatively short period in US history, transsexuality, a category that had once not existed, became a widely recognized term. It was named and described in the 1960s in influential publications, including Harry Benjamin's The Transsexual Phenomenon (1969).

> The national picture changed from one of no significant institutional support for transsexual therapy and surgery in 1965 to a situation in 1975 where about twenty major medical centers were offering treatment and some thousand transsexuals had been provided with surgery. Historians of transsexuality have been somewhat dazzled by this demonstration of the making of sex. One of the most perceptive observers of the twentieth-century historical sociology of sex has written of transsexualism's "widespread public and professional acceptance" by the 1970s, "an accepted syndrome, buttressed by a vast medical armamentarium of research, publications, and treatment programs." The result is not exactly a case of hidden history but rather inattention to an important period of critique, and the implied success of systems of technology and therapy that I am going to suggest were far more tentative, contested, and fragmentary. This neglected story both rethinks the early history of a new medical diagnosis and entity and sheds rather negative light on US psychiatric and surgical practices in the 1960s and 1970s."

- The Treatment of Adolescent Transsexuals: Changing Insights.
    - "Treatment of individuals with gender identity disorder (GID) has in medicine nearly always met with a great deal of skepticism. Professionals largely follow the Standards of Care of the World Professional Association for Transgender Health. For adolescents, specific guidelines have also been issued by the British Royal College of Psychiatrists."
- Brain Gender Identity - a presentation by Dr Sidney Ecker, MD FACS
    - Associated Presentation
- Bigender and the Brain
    - "CG Gender Identity is also set by pre-natal influences. The effects appear later, and the tell-tale neurology appears after Gender Identity is formed. But the pattern that dictates the later neurological development is just as pre-ordained. It is a more subtle effect that sexual orientation, and many people are essentially Bi-Gendered, and would be able to function in either a male or female role, as circumstances dictate. Relatively few are strongly gendered, but for those that are, it's unchangeable. Just as CG sexual orientation is strongly correlated with CG **behaviour** in children, CG gender identity is universally(?) associated with CG **patterns of thought** in children."
- Sex Reassignment. Thirty Years of International Follow-up Studies After Sex Reassignment Surgery: A Comprehensive Review, 1961-1991
    - "The individual studies and reviews are annotated by the authors, and are discussed within the frame of the development of the field. Sex reassignment, properly indicated and performed, has proven to be a valuable tool in the treatment of individuals with transgenderism."
- Current Thinking on the Etiology of Gender Dysphoria reprinted from The Gendered Self--Further commentary on the transsexual phenomenon
    - "There is no clearly understood cause for gender variance. However, we have enough information about fetal brain development and the procedure the embryo goes through in becoming either male, female or intersexed, to implicate the complexity of the procedure itself as a cause of the spontaneous sex reversal or potential sex/gender discontinuity (1). What follows is an abbreviated sample of what we now know about what goes on relative to being gendered physiologically."
- Biased-Interaction Theory of Psychosexual Development: "How Does One Know if One is Male or Female?"

- "A theory of gender development is presented that incorporates early biological factors that *organize* predispositions in temperament and attitudes. With *activation* of these factors a person interacts in society and comes to identify as male or female. The predispositions establish preferences and aversions the growing child compares with those of others. All individuals compare themselves with others deciding who they are like (*same*) and with whom are they *different*. These experiences and interpretations can then be said to determine how one comes to identify as male or female, man or woman. In retrospect, one can say the person has a gendered brain since it is the brain that structures the individual's basic personality; first with inherent tendencies then with interactions coming from experience."

- Gender Differences in Human Brain: A Review
  - "Why do men and women think differently? Why do they behave differently in stressed situations? Why do women act more emotionally as compared to men? Why do men and women excel at different types of tasks? Why do boys like to play with cars and trucks and superman? These are the common questions which arise commonly in minds.The human brain is a highly complex organ. Studies of perception, cognition, memory and neural functions have found apparent gender differences. These differences may be attributed to various genetic, hormonal, and environmental factors and do not reflect any overall superiority advantage to either sex. Both sexes are equal in intelligence, but tend to operate differently. Men and women appear to use different parts of the brain to encode memories, sense emotions, recognize faces, solve certain problems and make decisions. Indeed, when men and women of similar intelligence and aptitude perform equally well, their brains appear to go about it differently, as if nature had separate blueprints. Sex differences in the brain may play a role in learning processes, language development, and progression of neurologically-based diseases. Sex differences need to be considered in studying brain structure and function and may raise the possibility of sex-specific treatments for neurological diseases. In this article it is reviewed that how does the brain of a male look and function differently from a female's brain, and what accounts for these differences?"