

**Department of Clinical Neuroscience
Karolinska Institutet, Stockholm, Sweden**

Sex Steroids and Gene Variants in Bipolar Disorder

**Anette Johansson
MBBS, FRANZCP**



Stockholm 2012

All previously published papers and figures were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by Larserics Digital Print AB

© Anette Johansson, 2012
ISBN 978-91-7457-859-1

ABSTRACT

Bipolar disorder is a chronic, cycling disorder affecting circa 2% of the population and whose pathophysiology is still largely unknown. It has been suggested that common treatments used for bipolar disorder have effects on the sex steroids yet sex steroids have also been shown to have widespread effects in the brain in systems of relevance to bipolar disorder. For example, DHEAS and progesterone have opposing effects on the glutamatergic and GABAergic systems essential for the regulation of other brain processes. The enzymes which are involved in the interconversions between these compounds are affected by a number of factors including polymorphisms in the genes coding for these enzymes. Papers 1-3 examine common polymorphisms in *AKR1C4*, *HSD3B2* and *SRD5A1*, their relationships with 8-9 am serum hormone concentrations as well as to retrospectively but systematically collected data on symptoms which in animal and human studies have shown a possibility of connection to neurosteroid abnormalities. All investigations including history taking was done in euthymic phase of bipolar 1 or 2 disorders and included all available sources of information such as chart review and third party information. In paper 1, men but not women had lower serum progesterone concentrations during euthymic phase of bipolar disorder if they had exhibited manic irritability as opposed to euphoric mania. A gene variant of *AKR1C4* was associated with lower progesterone concentrations and polymorphisms of this gene were also more frequent in the males with prior irritability during mood elevation. Paper 2 showed that these same polymorphisms that increased risk in men *reduced* the risk for paranoid ideation during mania in women. However in men, DHEAS and progesterone were both lower and this coupled at a trend level to a polymorphism in the *HSD3B2* gene. In paper 3, depressive psychomotor agitation was coupled in both men and women to polymorphisms in *SRD5A1*. Yet only in men did one of the polymorphisms correspond to increased progesterone, a finding which was in line with the finding of higher progesterone if men had showed depressive psychomotor agitation.

Testosterone has different effects to progesterone and DHEAS in the brain and has been implicated in psychosis, having effects on the dopamine system. In paper 4, parameters measuring testosterone effects at different points in development were investigated in women with bipolar disorder who had exhibited psychotic features and compared with those in women with bipolar disorder without such features as well as healthy control women. The A allele at rs6152 of the Androgen Receptor gene was associated with a greater risk of having had psychotic symptoms and was at a trend level associated with an earlier debut of psychotic symptoms. The D2:D4 finger ratio which is believed to be a measure of prenatal testosterone milieu correlated with age of onset of psychosis indicating that high testosterone in the foetus predisposed the individual to an earlier psychosis onset but not to getting psychotic per se. A possible gene by drug interaction was found whereby the G allele rs6152 was associated with much higher bioavailable testosterone in the presence of neuroleptics. Upon further investigation this was explained by lower SHBG (sex hormone binding globulin) even in the presence of low doses of atypical neuroleptics.

Taken together these studies suggest the involvement of sex steroids in mediating risk to particular phenotypes of presentation of bipolar disorder, rather than bipolar disorder itself. The symptoms correspond to those regarded to be of the so called mixed states. Whilst the studies are of reasonable size in ascertaining hormonal differences the studies are small with respect to the genetic data and need to be replicated in larger samples.

SAMMANFATTNING PÅ SVENSKA

Bipolärt syndrom är en kronisk men cyklisk sjukdom som drabbar cirka 2 % av befolkningen och vars patofysiologi fortfarande till stora delar är okänd. Vissa läkemedel som används mot bipolärt syndrom påverkar könssteroiderna. Men könssteroider har även visats ha omfattande effekter på hjärnsystem av relevans för bipolärt syndrom. Exempelvis har könssteroider som DHEAS och progesteron visats ha motsatta effekter på de glutamaterga och GABAerga systemen som är nödvändiga för regleringen av andra hjärnprocesser. Enzymerna som är involverade i omvandlingen av könssteroiderna påverkas av många olika faktorer av vilka korresponderande genetiska polymorfismer antas vara en. I delarbete 1-3 undersöks polymorfismer i *AKR1C4*, *HSD3B2* och *SRD5A1* och deras relation till morgonkoncentrationer av hormoner i serum samt till retrospektivt insamlade data på symptom som i djur- och humanstudier kan vara kopplade till neurosteroidsystemet. Alla utredningar inklusive anamnestagning skedde under eutym fas av bipolär 1 eller 2 syndrom. Flera informationskällor för skattning av symptom användes vilket ökar reliabiliteten. I delarbete 1 fann vi att män, men inte kvinnor, hade lägre serumprogesteron om man tidigare uppvisat irritabilitet istället för eufori under manier. En genvariant i *AKR1C4* var associerad lägre serumprogesteron och den varianten var vanligare hos männen, men inte kvinnorna, som uppvisat manisk irritabilitet. Delarbete 2 visade att den *AKR1C4* allel som hos män var associerat till manisk irritabilitet, var hos kvinnor skyddande för misstänksamhet under mani. Hos männen var både DHEAS och progesteron lägre hos dem med misstänksamhet under mani och detta var på trendnivå kopplat till polymorfismer i *HSD3B2* genen. I delarbete 3 fann vi en association mellan psykomotorisk agitation (rastlöshet) under depressioner och vissa genvarianter i *SRD5A1* hos både män och kvinnor. Men enbart hos männen var risk polymorfismerna och/eller depressiv agitation associerat med högre serumprogesteron.

Testosteron har delvis andra effekter än progesteron och DHEAS i hjärnan och har tillskrivits betydelse för psykos. I delarbete 4 studerades flera parametrar som speglar testostereffekten hos kvinnor med bipolär 1 eller 2 syndrom som antingen uppvisat psykotiska symptom eller ej och friska kvinnor. A allelen av rs6152 i androgenreceptorn var associerad med en högre risk för tidigare psykotiska symptom och var på en trendnivå associerad med tidigare debut av psykosymptom. Mer maskulin fingerlängdskvot (D2:D4), ett mått på prenatal testosteronexponering, korrelerade med tidig debut av psykosymptom men inte till psykos *per se*. En gen-läkemedelinteraktion påvisades där den lägre risk G allelen av rs6152 var associerad till mycket högre bioaktivt testosteron via lägre SHBG (sex hormone binding globulin) och med låga doser av atypiska neuroleptika.

Sammantaget talar dessa studier för att könssteroider har betydelse för det fenotypiska uttrycket av bipolärt syndrom. De studerade symptomen ingår i det konstrukt som benämnes blandade episoder. Studierna har adekvat storlek för de hormonella fynden men är små avseende de genetiska fynden vilka därför bör bekräftas i större grupper.

LIST OF PUBLICATIONS

- I. Johansson, A.G., Nikamo, P., Schalling, M., Landén, M., 2011.
AKR1C4 gene variant associated with low euthymic serum progesterone and a history of mood irritability in males with bipolar disorder.
Journal of affective disorders. Sept.133, 346-351.
- II. Johansson, A.G., Nikamo, P., Schalling, M., Landén, M., 2012.
Polymorphisms in AKR1C4 and HSD3B2 and differences in serum DHEAS and progesterone are associated with paranoid ideation during mania or hypomania in bipolar disorder.
Eur Neuropsychopharmacol. Aug 22, 9, 632-640
- III. Johansson, A.G., Nikamo, P., Schalling, M., Landén, M.
Depressive psychomotor agitation in bipolar patients is associated with variants of the SRD5A1 gene and higher progesterone concentrations in men.
(submitted)
- IV. Johansson, A.G., Westberg, L., Ekman, C-J., Sellgren, C., Landén, M.
Measures of testosterone function in relation to psychosis in women with bipolar disorder.
(submitted)

TABLE OF CONTENTS

PROLOGUE	5
1 INTRODUCTION	6
1.1 Historical Concept of Bipolar Disorder	6
1.2. Modern Definition and Classification of Bipolar Disorder	8
1.2.1 Symptom Analysis and Stability	11
1.2.2 The Dilemma of Mixed States	12
1.2.3 The Effect of Temperament on Symptomatology During Mood Episodes	16
1.2.4 Establishing Phenotypes for Genetic Analysis	16
1.3 Neurosteroids	17
1.3.1 Concepts	17
1.3.2 Biosynthetic Pathways	17
1.3.2.1 <i>3β-Hydroxysteroid Dehydrogenase (HSD3B1 or 2 also known as 3βHSD)</i>	18
1.3.2.2 <i>Steroid-5-alpha-reductase (SRD5A1)</i>	19
1.3.2.3 <i>Aldoketoreductase (AKR1C, also known as 3α-Hydroxysteroid Dehydrogenase Type 1 as well as 3-AHSD as well as 3αHOR)</i>	22
1.3.3 DHEA and DHEAS	23
1.3.3.1 <i>Factors Influencing Synthesis and Metabolism</i>	23
1.3.3.2 <i>Role in Neurogenesis and Neuronal Survival</i>	25
1.3.3.3 <i>Effects on Neurotransmission and the Catecholamine System</i>	25
1.3.3.4 <i>Sigma Receptor Effects</i>	26
1.3.3.5 <i>Other Effects in the Brain</i>	28
1.3.3.6 <i>Findings Related to Psychiatric Disorders and Behaviour</i>	28
1.3.4 Progesterone and Allopregnanolone	31
1.3.4.1 <i>Factors Affecting Synthesis and Metabolism of Progesterone and Allopregnanolone (Allo)</i>	31
1.3.4.2 <i>Effects in the Brain by Progesterone</i>	32
1.3.4.3 <i>The Progesterone Receptor</i>	33
1.3.4.4 <i>Progesterone – Psychiatric Disorders and Behaviour</i>	34
1.3.4.5 <i>Effects of Allopregnanolone in the Brain</i>	34
1.3.4.6 <i>Allopregnanolone – Psychiatric Disorders and Behaviour</i>	35
1.3.5 Testosterone	36
1.3.5.1 <i>Factors Affecting Testosterone Concentrations</i>	36
1.3.5.2 <i>Effects in the Brain</i>	38
1.3.5.3 <i>The Androgen Receptor</i>	39
1.3.5.4 <i>D2:D4 Digit Ratio and Prenatal Testosterone Exposure</i>	40
1.3.5.5 <i>Associations with Behaviour and Psychiatric Disorders</i>	41
1.3.6 Summary of Neurosteroid Effects	45
2 AIMS OF STUDIES	47
3 METHODS	48

3.1	Overview of Subject Selection and Assessments	48
3.2	Ethics of Study	50
3.3	Specific Methodology Studies 1-4	50
4	RESULTS	53
4.1	Study 1	54
4.2	Study 2	55
4.3	Study 3	56
4.4	Study 4	58
5	DISCUSSION AND IMPLICATIONS FOR FUTURE RESEARCH	61
5.1	Studies 1-3	61
5.2	Study 4	63
5.3	Methodological Considerations:	64
6	CONCLUSION	67
7	ACKNOWLEDGEMENTS	68
8	REFERENCES	70

ABBREVIATIONS

5 α DHDOC	5-alpha-dihydrodeoxycorticosterone
5HT	Serotonin
ADE	Affective disorders evaluation
AKR1C	Aldoketoreductase
AKR1C2	Aldoketoreductase type 3
AKR1C3	Aldoketoreductase type 2
AKR1C4	Aldoketoreductase type 1
ALLO	Allopregnanolone
ANK3	Ankyrin 3
AR	Androgen receptor
AUDIT	Alcohol use disorders identification test
BDNF	Brain derived neurotrophic factor
BNST	Bed nucleus stria terminalis
CACNA1C	Calcium channel voltage gated dependent p/q type α -1 subunit
CAH	Congenital adrenal hyperplasia
CSF	Cerebrospinal fluid
D1 receptor	Dopamine 1 receptor
D2:D4	2 nd digit to 4 th digit ratio
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate
DHEA(S)	Dehydroepiandrosterone and Dehydroepiandrosterone sulphate
DHT	Dihydrotestosterone
DISC1	Disrupted in schizophrenia 1
DSM	Diagnostic and statistical manual of mental disorders (versions follow in roman numerals, except for version 5)
DUDIT	Drug use disorders identification test
ECT	Electroconvulsive therapy
EEG	Electroencephalogram
ER	Endoplasmic reticulum
FSH	Follicle stimulating hormone
GABA _A	Gamma aminobutyric acid type A
GFAP	glial fibrillary acidic protein
GnRH	Gonadotrophin releasing hormone
GWAS	Genome wide association studies
HSD3B1/2	3 β -hydroxysteroid dehydrogenase type 1/ or type 2
HVA	Homovanillic acid
ICD	International Classification of Diseases (followed by version number)
IL-1 β	Interleukin 1- β
IL-10	Interleukin 10
LH	Luteinizing hormone
MADRS	Montgomery-Åsberg depression rating scale
MAP2	Marker microtubule associated protein-2
MAPK	Mitogen activated protein kinase
MINI	Mini-international neuropsychiatric interview
MMSE	Mini mental state examination
NGFIB	Nerve growth factor induced clone B
NMDA	N-methyl-D-aspartate
OFC	Orbitofrontal cortex
PCOS	Polycystic ovarian syndrome

PMDD	Premenstrual dysphoric disorder
PFC	Prefrontal cortex
PR	Progesterone receptor
PTSD	Posttraumatic stress disorder
SCID-II	Structured clinical interview for DSM axis 2 diagnosis – screening
SHBG	Sex hormone binding globulin
SNP	Single nucleotide polymorphism
SRD5A1 or 2	Steroid 5 α -reductase type 1 or type 2
SSP	Swedish scales of personality
STEP-BD	Systematic treatment enhancement programme for bipolar disorder
SULT2A1	DHEA sulphotransferase
THDOC	tetrahydrodeoxycorticosterone
TNF α	Tumour necrosis factor-alpha
YMRS	Young mania rating scale

PROLOGUE

"Why do women who have had a postpartum psychosis become hairy?"

(citation from a mother of one who had developed male patterned hirsutism following a postpartum psychosis some years earlier)

This was the question posed to me, a first year resident in psychiatry in Perth, Western Australia which spawned my interest in understanding the links between the sex-hormones and the psyche. At the time it was thought that neuroleptics stimulating prolactin release activated the hypothalamic.pituitary axis and could thus cause male patterned hirsutism. Yet this woman indicated that the hair growth started *days* after becoming psychotic, before being given neuroleptics and remained long after the neuroleptics had been withdrawn (at that time some 15 years). The explanation of neuroleptic induced changes seemed not to be the whole story...

1 INTRODUCTION

1.1 Historical Concept of Bipolar Disorder

Bipolar disorder used to be included in the group of illnesses called “the psychoses” which included not only bipolar disorder (known then as manic-depressive illness) but also schizophrenia, the paraphrenias and the various organic psychoses for example epilepsy, neurosyphilis and poisonings from for example arsenic or bromide (Jaspers, 1942). Kraepelin, whilst advocating a separate classification of manic depressive illness from schizophrenia on the basis of course and prognosis, conceded that “to decide whether an isolated state belongs to manic depressive insanity or not without a survey of the whole course, is not always easy. The principal difficulties arise in general with paralysis (tertiary syphilis) and dementia praecox (schizophrenia)”. He advised that in order “to decide between the two diseases (schizophrenia and bipolar disorder) one needed to consider their “history of origin”. By this he meant the age of onset, if “manic or cyclothymic predisposition” was present and, to a certain extent, the type of family history (whether bipolar disorder, unipolar depressions or schizophrenia predominated) (Kraepelin, 1921). Kraepelin saw manic depressive insanity as a much more episodic illness with full recovery between episodes in contrast to those with dementia praecox (later to be termed schizophrenia) which was a chronic unremitting condition. However he noted that 10% of persons with manic depressive illness developed a chronic course which he labelled as manic dementia. (Kraepelin, 1921). The Kraepelinian viewpoint was continued in DSM-II (Diagnostic and Statistical Manual for Mental Disorders- version II) and ICD-9 (International Classification of Diseases version 9) where bipolar disorder or manic depressive illness as it was then known was still included in the psychoses (APA, 1968; WHO, 1977). Of interest in the Kraepelinian classification is that many of those with a schizophrenic like picture but fully remitting would have been considered to have another diagnosis especially manic depressive illness.

Psychosis as a concept has waxed and waned in popularity. What seems on the surface as self-evident – that of an inability to interpret and to deal with reality in an adaptive way – becomes murky when attempting to define the conceptual components. The definition in Kaplan and Sadock 8th edition (Sadock, 2005) is “a mental disorder in which the thoughts, affective response, ability to recognize reality and ability to communicate and relate to others are sufficiently impaired to interfere grossly with the capacity to deal with reality; the classical characteristics of psychosis are impaired reality testing, hallucinations, delusions and illusions”. The description clearly specifies more symptoms than the examples given: for instance, very elevated or very low moods can markedly affect the interpretation of events whilst thought disorder directly impairs the capacity to communicate. By this definition bipolar disorder can in its severe form be regarded as a psychotic illness.

The definition of psychosis requires a judgement of severity of the functional impairments associated with the symptoms, a subjective task with inherent difficulties. This latter point also raises the possibility of those having these same symptoms not automatically being impaired by them and thus not being considered psychotic. For example, those who get on with life despite a continual running commentary by a voice – are they to be regarded as psychotic or not? The imprecision in the psychosis concept leads to the possibility of idiosyncratic interpretation of the concept. In fact inter-rater reliability of the concept was found to average 0.55 in a number of studies (Spitzer and Fleiss, 1974). As a result, psychosis as a concept was not seen as a helpful to retain in the DSM-III or in the ICD-10 whose ambitions were to operationalize symptom definitions so as to make the inter-rater

reliability of diagnoses higher (Spitzer et al., 1975; Spitzer et al., 1979). Alas “psychotic disorder” remained in some diagnostic titles but now without an accompanying full definition of the concept, being reduced instead to criteria of hallucinations, delusions, disorganized speech and disorganized or catatonic behaviour as in schizophrenia (APA, 1980; WHO, 1993). Bipolar disorder, which often but not always, presents with psychotic features, was thereby excluded from the psychotic disorders group and moved into a separate affective disorders category.

The debate as to whether bipolar disorder belongs to the psychotic disorders group or to a separate mood disorder group has once again been actualised in the lead up to the DSM-5 and ICD-11. Whilst the study group charged with examining evidence for shared features across diagnostic groups have opted for conserving the current demarcation of DSM-IV-tr many researchers have advocated for the return of the bipolar 1 diagnosis to the “psychotic disorders” (Bora et al., 2009; Carpenter and Fischer, 2009; Craddock et al., 2009; Craddock and Owen, 2005; Jabben et al., 2009; Murray et al., 2004; Owen et al., 2007; Zanelli et al., 2010). Advocates of a unitary mood disorder classification have wished to retain bipolar 1 and 2 subtypes together and argued for the differences seen between schizophrenia and bipolar disorder. Differences between bipolar disorder and schizophrenia have been found for example in the neurodevelopmental pathway where schizophrenia is associated with greater prevalence of winter births, delivery complications and neuromotor features during childhood than bipolar disorder (Murray et al., 2004). This has previously been thought to associate with foetal infection rates however epidemiological studies examining infectious epidemics and frequency of outcome in form of bipolar disorder are lacking and the above hypothesis is yet to be proven. Studies examining presence of antibodies to infectious agents such as toxoplasmosis or cytomegalovirus or herpes do tend to show increased prevalence of antibodies in bipolar disorder just as they do for schizophrenia (Pearce et al., 2012; Tedla et al., 2011) even though not all studies have been able to confirm this (Mortensen et al., 2011) leading one to conclude that there are other factors responsible for the noted differences. Bipolar disorder patients tend to do better than schizophrenia patients in verbal measures of neuropsychological tests and possibly also have higher premorbid intellectual function although those with psychotic symptoms tend to equate more with the schizophrenia group (Daban et al., 2006). Great heterogeneity exists however between patients even within the same diagnostic group. Whilst there are similarities in the longitudinal development of brain changes including brain volumes and ventricular enlargement there are differences also: cortical thinning appears to be a feature of schizophrenia and not bipolar disorder (Rimol et al., 2012) and some areas appear to enlarge during periods of illness in bipolar disorder but not so in schizophrenia (Fornito et al., 2009). Brain network disconnectivity differs such that the meso-paralimbic to fronto-temporal network (a network involved in emotional processing) is abnormal in bipolar disorder and not in schizophrenia (Meda et al., 2012). The localization of abnormal resting default modes in intrinsic brain networks differ also between schizophrenia and bipolar disorder whilst the low power of low frequency rhythms is found equally in the two patient groups but more often than in healthy controls (Calhoun et al., 2012).

Over the past 20 years an ever increasing body of evidence suggests overlap of shared genetic loading between schizophrenia and bipolar disorder. This has been seen in both large epidemiological studies (Lichtenstein et al., 2009) as well as with regard to polymorphisms in genes such as disrupted in schizophrenia-1 (DISC1) (Hodgkinson et al., 2004; Lepagnol-Bestel et al., 2010; Maeda et al., 2006; Millar et al., 2000; Perlis et al., 2008), ankyrin3 (ANK3) and calcium channel, voltage dependent p/q type alpha 1 subunit (CACNA1C)

(Baum et al., 2008; Ferreira et al., 2008; Scott et al., 2009; Sklar et al., 2008; Smith et al., 2009). With regard to symptom classification of psychosis as opposed to syndrome classification, brain derived neurotrophic factor (BDNF) has shown some overlap (Baum et al., 2008; Liu et al., 2008b; Neves-Pereira et al., 2005; Neves-Pereira et al., 2002; Sklar et al., 2002). However some genes appear to be more specific for bipolar disorder and, not surprisingly given the cyclicity of the illness, various “clock” genes appear to be amongst these (Baum et al., 2008; McGrath et al., 2009; Shi et al., 2008; Sklar et al., 2008). The search for mechanisms linking polymorphisms to disease findings have inspired a number of studies looking at age of onset, severity ratings, brain morphology, brain activation and neurophysiological differences according to genotype as well as attempting to couple individual symptoms with genotype (see for example with regards to BDNF. (Agartz et al., 2006; Numata et al., 2006; Spalletta et al., 2010; Whalley et al., 2010; Xu et al., 2008)

With regard to the depressive spectrum disorders with which bipolar disorder nowadays is classified it is acknowledged that there are major differences between bipolar disorder and unipolar depression in terms of imaging findings, (Goldberg et al., 2009a) prevalence of antibodies to infectious agents (Pearce et al., 2012) and in neurocognitive profiles (Castaneda et al., 2008; Gualtieri and Morgan, 2008) even though very few studies have directly compared euthymic bipolar patients with euthymic patients suffering from recurrent depressions. The distinction between bipolar 1 and 2 disorders has been questioned given the overlap of cognitive deficits (see meta-analysis by Bora et al (2010)) and symptoms which only differ in severity and degree of functional impairment.

1.2. Modern Definition and Classification of Bipolar Disorder

DSM-IV-tr defines bipolar disorder as consisting of bipolar 1 disorder, bipolar 2 disorder, cyclothymia and bipolar disorder not otherwise specified. In this discussion, focus will be placed on the first two of these. The criteria for bipolar 1 and 2 disorders are listed in Table 1. In Bipolar 1 disorder there has been at least 1 manic or mixed episode but there may or may not have been depressive episodes whereas in bipolar 2 disorder there have been depressive episodes and periods of hypomania. These symptoms are the same as for mania but which do not *markedly impair functioning, cause hospitalization or include psychotic symptoms*. In other words these states differ only in severity and not in character. DSM-IV-tr added to the previous definition in DSM-IV that the symptoms of hypomania may indeed improve functioning, a fact that is well known clinically where bursts of creativity, heightened clarity

Table 1: Criteria for Bipolar Disorder in DSM-IV-tr		
Bipolar 1 disorder		Bipolar 2 disorder
Single manic episode	History of at least 2 mood episodes 1 of which must be either of manic or mixed character , not solely hypomanic. Depressive, hypomanic episodes may or may not have been present	One or more depressive episodes – never had a manic episode. One or more hypomanic episodes
The symptoms cause marked distress or impairment in social, occupational or other important areas of functioning and may require hospitalization and may include psychotic features		The symptoms cause significant but not marked distress or impairment in social, occupational or other important areas of functioning and do not necessitate hospitalization nor should a hypomanic episode include psychotic features
Symptoms not better accounted for by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder or psychotic disorder not otherwise specified, or other organic disorders be they substance related or due to medical condition		

and energy often help affected individuals to focus on desired endeavours. Table 2 describes the symptom criteria for each type of episode. Persons not meeting the time criteria for symptom complexes are in the DSM system diagnosed with either cyclothymia or bipolar disorder not otherwise specified. This time criterion is controversial (Akiskal et al., 2000; Angst et al., 2003; Lewinsohn et al., 2002) with the suggestion of even 1-2 days of hypomania being indicative of a similar disease process to that of longer duration episodes. Psychotic symptoms are not specifically mentioned in the criteria for mania or depression in the DSM-IV-tr.

ICD-10 defines bipolar disorders differently to DSM-IV-tr. In particular it does not differentiate bipolar 1 from bipolar 2 even though it specifies differences between manic and hypomanic episodes.

- 1) In mania, symptoms of increased libido and sexual indiscretions are singled out as a separate criterion from other reckless behaviours; inappropriate social behaviour with loss of inhibitions is also made a separate item
- 2) Mania is subdivided into (i) “nonpsychotic” mania which may include hyperacusis and the appearance of vivid colours, and (ii) “psychotic” which is further divided into mood-congruent (grandiose) and non mood-congruent (voices speaking about neutral subjects, delusions of reference or persecution).
- 3) All delusions of control, passivity phenomena and other bizarre delusions as well as critical 3rd person hallucinations places the person in a schizoaffective category which in ICD-10 does not require periods of separate mood and schizophrenic like episodes as this does in DSM-IV-tr. By contrast DSM-IV-tr places bizarre delusions including those of control in the mood incongruent category of psychotic, severe mania (see Table 3)
- 4) Hypomania excludes grandiosity and racing thoughts as symptom criteria but adds increased libido, mild overspending and recklessness as well as increased sociability or irresponsible behaviour thereby clearly separating out those that are moving toward psychotic experiences that may be partially controlled by present treatment.
- 5) ICD 10 does not specify required numbers of symptoms of opposite pole to be present in order to diagnose a mixed episode but regards rapid shifts between manic, depressive and hypomanic episodes as important along with a 2 week minimum period. This means it is easier to meet criteria for mixed episode than it is in the DSM classification and that some of these will be regarded as having rapid cycling variety of bipolar disorder in the DSM tradition as opposed to mixed episodes.

Table 3: Key Differences Between DSM-IV-tr and ICD-10 with Respect to Diagnosis of Affective Psychotic Symptoms		
Symptoms	ICD-10	DSM IV-tr
Delusions of persecution	Mood incongruent	May or may not be mood incongruent depending upon whether they relate to grandiosity
Neutral voices during manic or depressive phase	Mood incongruent	Mood incongruent
Bizarre delusions, delusions of control, passivity phenomena and all 3 rd person voices commenting on person	Schizoaffective disorder even if they occur during affective episode	Mood incongruent

Table 2: DSM-IV-tr Mood Episode Definitions				
Manic Episode	Hypomanic Episode	Mixed Episode	Depressive Episode	
A distinct period of abnormally and persistently elevated, expansive or irritable mood > 1 week or less if hospitalized	A distinct period of persistently elevated, expansive or irritable mood lasting throughout at least 4 days	Criteria are met for both a manic episode and a depressive episode nearly everyday during at least 1 week	Either depressive mood OR loss of interest for at least 2 weeks – may have both	
At least 3 (and 4 if mood irritable) of the following symptoms 1) inflated self esteem, grandiosity 2) decreased need for sleep 3) more talkative than usual or pressure to keep talking 4) flight of ideas or racing thoughts 5) distractibility 6) increase in goaldirected behaviour or psychomotor agitation 7) excessive involvement in pleasurable activities that have a high potential for painful consequences (eg unrestrained buying, sexual indiscretions, high risk business ventures)	At least 3 (and 4 if mood irritable) of the following symptoms 1) inflated self esteem, grandiosity 2) decreased need for sleep 3) more talkative than usual or pressure to keep talking 4) flight of ideas or racing thoughts 5) distractibility 6) increase in goaldirected behaviour or psychomotor agitation 7) excessive involvement in pleasurable activities that have a high potential for painful consequences (eg unrestrained buying, sexual indiscretions, high risk business ventures)	Symptoms from mania and depression	In addition – at least 3-4 of following 1) weight loss or gain or increase or decrease in appetite 2) increased or decreased sleep 3) observable psychomotor slowing (retardation) or restlessness (agitation) 4) fatigue or loss of energy 5) feelings of worthlessness, or guilt which may be delusional 6) concentration difficulties and indecisiveness 7) recurrent thoughts of death and suicidality	
The symptoms do not meet criteria for a mixed episode	The episode is associated with an unequivocal change in functioning that is uncharacteristic of the person when not symptomatic and is observable by others		The symptoms do not meet criteria for a mixed episode	
Causes marked impairment in occupational functioning or in usual social activities or relationships with others, or necessitates hospitalisation to prevent harm to self or others or there are psychotic features	The episode is not severe enough to cause marked impairment in social or occupational functioning nor necessitate hospitalisation nor are there psychotic symptoms. In fact there may be improved functioning	Causes marked impairment in occupational functioning, in usual social activities or relationships with others, or necessitates hospitalisation to prevent harm to self or others or there are psychotic features	The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning	
The symptoms are not due to a medical condition				
			Not accounted for by uncomplicated bereavement	

Thus in conclusion, these differences highlight the uncertainty in operationally classifying what appear to be spectrum conditions into categorical entities. Differences in diagnostic systems do however impact prevalence estimates of bipolar disorder and its subtypes (Angst et al., 2003; Vieta and Morralla, 2010) as well as phenotypes used in delineating the illness for further study potentially aiding or hampering progress in understanding the pathophysiological basis of bipolar disorder.

1.2.1 Symptom Analysis and Stability

Factor analytic studies have addressed a core question in attempting to define useful phenotypes of bipolar disorder for biological research – that of whether certain symptoms cluster together in mania or depression. Table 4 summarizes the studies that have been conducted over the past 15 years. A variety of rating scales have been used in the different studies making direct comparisons difficult. By and large however there appear to be some common themes in mania i) core hedonic features, ii) increased psychomotor activity, iii) dysphoria

Table 4: Factor analytic studies of mania and bipolar depression			
Mania			
Study	Number of participants	Rating scale	Factors
Cassidy et al., 1998	81	SMNS-20	5 (dysphoric, psychomotor pressure, psychosis, increased hedonic function, irritable aggression)
Serretti et al., 1999	523	Opcrit 16	3 (psychomotor excitement, psychosis, irritability/motor agitation)
Dilsaver et al., 1999	105	SADS-37	4 (manic activation, depressed state, sleep disturbance, irritability/paranoia)
Rossi et al., 2001	124	BRMaS-11 BRMeS-11	5 (activation-euphoria, depression, psychomotor retardation, hostility, sleep disturbance)
Swann et al., 2001	162	SADS, ARDS	6 (impulsivity, hyperactivity, anxious pessimism, distressed appearance, hostility, psychosis)
Perugi et al., 2001	153	CPRS-18	5 (depressive, irritable agitated, euphoric, grandiose, accelerated, paranoid - anxious)
Sato et al., 2002	576	AMDP-37	7 (depressed mood, irritable aggression, insomnia, depressive inhibition, pure manic symptoms, emotional lability/agitation, psychosis)
Gonzales-Pinto et al., 2003	103	YMRS-11 HDRS-21	5 (depression, dysphoria, hedonism, psychosis, activation)
Akiskal et al., 2003	104	MSRS-26 HDRS-17	7 (disinhibition, paranoia-hostility, deficit, grandiosity-psychosis, euphoria, depression, hypersexuality)
Picardi et al., 2008	88	BPRS-24	4 (mania, disorganization, positive symptoms, dysphoria)
Gupta et al., 2009	225	CASH	6 (psychosis, irritability/aggression, dysphoria, accelerated thought stream, hedonia, hyperactivity)
Bipolar depression			
Benazzi et al., 2001	251	MADRS	3 (sadness, reduced sleep and appetite, hopelessness/lassitude/concentration and inability to feel)
Harvey et al., 2009	134	DPRS-17	5 (psychosis/retardation, somatisation/phobia/euphoria, hostility/excitement, obsessions/disorganization, interpersonal sensitivity/anxiety)
ADRS – Affective Disorders Rating Scale; AMDP – Association for Methodology and Documentation in Psychiatry; BPRS – Brief Psychiatric Rating Scale; BRMaS – Bech-Rafaelsen Mania Scale; BRMeS – Bech-Rafaelsen Melancholia Scale; CASH – Comprehensive Assessment of Symptoms and History; CPRS – Comprehensive Psychopathology Rating Scale; DPRS – Derogatis Psychiatric Rating Scale; HDRS – Hamilton Depression Rating Scale; MADRS – Montgomery-Åsberg Depression Rating Scale; Opcrit – Operational criteria checklist for psychotic illnesses; SADS – Schedules for Affective Disorders and Schizophrenia; SMS – Scale for Manic States; YMRS - Young Mania Rating Scale			

during mania separating out from other manic symptoms, iv) irritability/hostility coupled in some studies with motor agitation and in others with paranoia, whilst v) paranoia itself appeared to be separate from grandiosity supporting the ICD distinction of paranoia as mood incongruent. This latter is interesting given the frequent clinical association between grandiosity (for example, being sent to achieve a particular mission, being a representative of God or some important person) and feelings of being thwarted by carers and others giving rise to mistrust and paranoid ideation.

In bipolar depression there were no clear cut commonalities between the 2 studies but both groups noted that the factor split varied between recurrent unipolar depressions and bipolar depressions (Benazzi, 2001; Harvey et al., 2009).

How stable are symptom clusters in mania and depression over episodes? Clinical lore has regarded symptoms as often recurring across episodes almost in a signature pattern for each individual. These observations spawned the interest in identifying individual early warning signs of mania and depression in order to help individuals and their families to manage the illness, this now being a recognized part of the routine clinical management of bipolar disorder. However, not many studies have specifically addressed how great the symptom concordance is between episodes. Cassidy (2002) reported on 77 patients with bipolar disorder and found correlations of 0.4 to 0.6 for summed subscale scores for manic psychosis, irritable aggression, hedonic activation and dysphoria across episodes as well as a correlation 0.35 for pure mania. Sato (2003b) likewise described similar moderate stability of manic dysphoria, irritable aggression, psychomotor inhibition, pure mania, emotional lability and psychosis across 4 episodes in 253 patients. Coryell (1994) examined depression subtype in 57 bipolar 1 patients and 76 bipolar 2 patients and found high likelihood of same depressive subtype on relapse (agitated/retarded, psychotic, “endogenous”). Perlis (2009) examined 583 persons with a bipolar 1 or 2 disorder enrolled in the Systematic Treatment Enhancement Programme for Bipolar Disorder (STEP-BD) project for depressive symptoms and found greatest symptom stability for psychomotor agitation, hypersomnia, suicidal ideation, guilt/rumination, impaired concentration, insomnia, and distractibility and psychotic symptoms (although the latter were rare in the studied sample). Even when controlling for anxiety, substance use and rapid cycling status these symptoms appeared stable across episodes. Most of these studies comment that there is the greatest similarity in symptomatology between contiguous episodes and that those episodes coming later share fewer symptoms with the index episode. So whilst there are correlations between symptom clusters these are not absolute.

1.2.2 The Dilemma of Mixed States

The classic symptoms of mania and depression can be seen analogously as turning the thermostat up or down. These include the over and underactivity states where speed of speech and thought as well as motor activity are affected. Furthermore this analogy can explain the level of interest expressed in daily life for example with food, sex and pleasurable activities. It is not unreasonable to think that so called clock genes and “thermostat” mechanisms may be involved in the aetiology of these symptoms. However it is less easy to understand irritability and non mood-congruent psychotic features from this perspective as well as the intrusion of for example mania into depression or depression into mania.

Whilst mixed states have been recognized since antiquity as separate entities to pure excitation and pure melancholia (Koukopoulos et al., 2007; Marneros, 2001) it is Kraepelin

and Weygandt that first furthered the conceptual thinking behind these states (Kraepelin, 1921; Weygandt, 1899). Both saw 3 main symptom axes that could alternate and occur in different combinations (Figure 1). In short these are i) mood – dysphoric to euphoric, ii) psychomotor activity – inhibited (retardation) to excessive (purposeful or nonpurposeful meaning agitation), and iii) thought – inhibited to intense. This conceptualization fell into disuse in the 20th century until the emergence of hard to treat depressions, the issue of switch into mania from antidepressants and the issue of rapid cycling mood states which caused a resurgence of interest in the issue of mixed states (Koukopoulos et al., 2007).

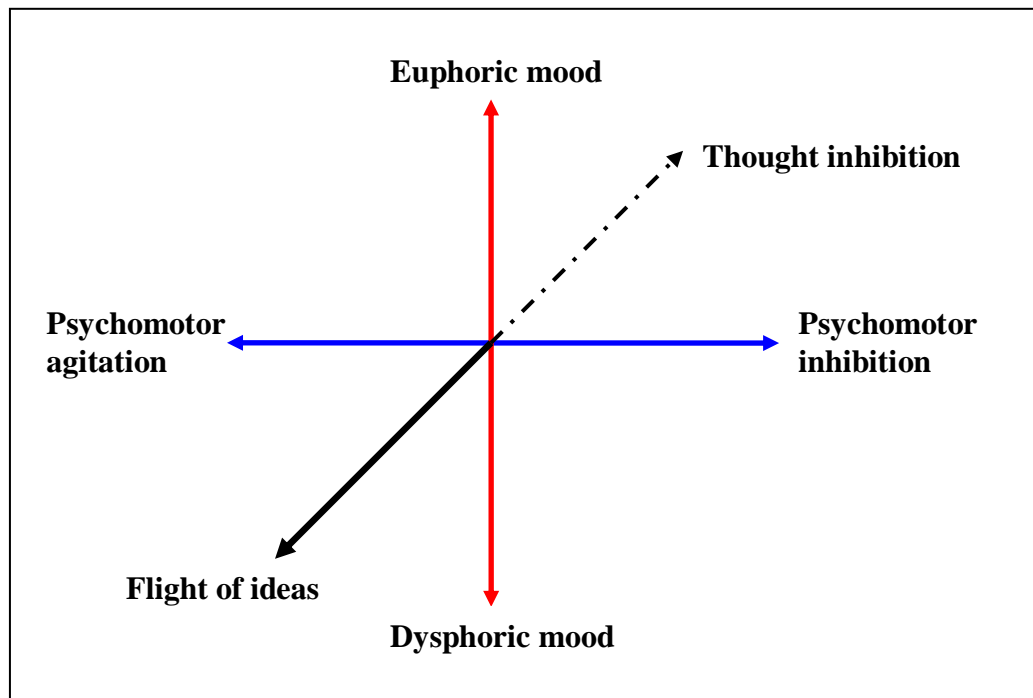


Figure 1: Kraepelinian conceptualization of the 3 axes which contribute to the manifestation of pure manic, depressive or mixed states

As regards to alternate descriptions of bipolar disorder including the mixed spectra of symptoms, Karl Leonard furthered the work of Wernicke, developing a nosology based on thousands of patient interviews. With an inter-rater reliability of 0.9 his nosology describes various subforms of pure mania and depression based on the traditional up or down-regulation of activity and emotional tone, as well as various forms of “cycloid psychoses” which he described as sharing traditional symptoms of manic-depressive illness including the longitudinal course of remitting episodes but were separate from the pure bipolar/unipolar disorders as well as separate to the schizophrenias. He described 3 types of cycloid psychosis i) anxiety-happiness psychosis which consists of frequent phases of anxiety, distrust, sense of being controlled and ideas of references which may alternate less frequently with periods of ecstatic mood and happiness, ii) excited-inhibited confusion psychosis whereby excitation and pressured speech are accompanied by incoherence, auditory hallucinations and misidentifications whilst periods of psychomotor inhibition may be accompanied by perplexity and ideas of reference, and iii) hyperkinetic-akinetic motility psychosis where excitable non-purposeful movements often are accompanied by pressure of speech and word salad and alternate with periods of psychomotor retardation and non-responsiveness. Implicit in his argument is the assumption of stability over time even though statistical data on the stability of these symptom clusters in the English translation of his nosology is lacking (Leonard, 1999). These forms bear some resemblance to the mood incongruent manic or

depressive episodes in the DSM-IV-tr even though aspects also relate to acute schizophrenia episodes and acute psychoses.

Perris (1988), amongst others, was inspired by this classification and operationalized a definition of cycloid psychosis (see Table 5). Comparing diagnoses made by British psychiatrists and those obtained by Perris using the descriptions of cycloid psychosis it was found that many of those diagnosed with schizophrenia and meeting criteria for cycloid psychoses ran a course akin to bipolar disorder and responded to electroconvulsive treatment (ECT) and lithium (Brockington and Roper, 1990). They were not akin to schizoaffective disorders (Brockington and Leff, 1979; Vogl and Zaudig, 1985) even though the ICD-9 drew this parallel. In the ICD-10 these are akin to acute polymorphic psychoses and in DSM-IV-tr they are the brief psychotic disorders and occasionally schizophreniform disorders with good prognostic features.

In the Kraepelinian tradition, disorganization and psychosis has been thought to reflect the severity of mania – corresponding to stage 3 of the illness with the more classical picture of acceleration and euphoria as stage 2 (Carlson and Goodwin, 1973) and hypomania being considered stage 1. Given the factor analytic studies and observations cited above it seems that either that the severity of the illness is relatively stable in the same individual over time, often despite episode modifying and prophylactic treatment, or that the differences reflect underlying biological systems differences.

DSM-IV-tr has, however, not adopted the Kraepelinian nor the Leonardian approaches to mixed states but adopted a very strict view on when to call a mood episode mixed – criteria must be met for both depression and mania concurrently. Mood incongruent psychotic symptoms are not specifically required although many authors consider this to be an important symptom occurring far more commonly in mixed states (Azorin et al., 2006; Dell'Osso et al., 1991). The literature distinguishes between mixed depressive states and mixed manic states (Perugi et al., 1997; Swann et al., 1993).

Mixed Mania or Dysphoric Mania:

Principal component analysis of episode defined mixed mania have yielded i) a depressive factor, ii) a dysphoria factor which included irritability, impulsivity, inner tension and distressing sensitivity to stimuli, and iii) an insomnia factor (Bertschy et al., 2007). Half of those with strictly defined mixed mania have psychotic features (Dell'Osso et al., 1993) the

Table 5: Perris' Criteria for Cycloid Psychosis
A. An acute psychotic condition with onset in patients ranging from 15-50 years
B. Sudden onset (hours to days)
C. 4 or more of the following <ul style="list-style-type: none"> 1) confusion, perplexity or puzzlement 2) mood-incongruent delusions often persecutory 3) hallucinations often related to themes of death 4) free-floating fear and anxiety 5) deep feelings of happiness or ecstasy often with religious colouring 6) motor disturbance – increased or decreased (retardation, agitation) 7) particular concern with death 8) mood swings
D. The symptoms change frequently during the same episode

majority of these non mood-congruent (Dell'Osso et al., 1991). Features of hostility and agitation as well as disorganization, impaired judgement and lack of insight have been found to be greater in mixed mania than in pure mania and in agitated depression (Swann et al., 1993). Most have had depressive episodes and are more likely to have future mixed episodes (McElroy et al., 1995). Mixed episodes have been suggested to be less responsive to lithium than to sodium valproate (Himmelhoch et al., 1976; Prien et al., 1988; Swann et al., 1997). The long-term course has been found by some to be no different to that of pure mania (McElroy et al., 1995) even when considering those with mood incongruent psychotic features (Gaudiano et al., 2007) yet others have found mixed states with mood incongruent mania to be associated with shorter periods of remission and lower psychosocial functioning (Azorin et al., 2006; Miklowitz, 1992; Strakowski et al., 2000; Tohen et al., 1990). Even mixed states without mood-incongruent psychotic features have in follow-up of first episode patients heralded worse outcome in some studies than pure mania (Baldessarini et al., 2010; Dodd et al., 2010; Salvatore et al., 2011).

Mixed Depression:

Depression with manic symptoms are most common in bipolar patients with rates of 36% compared with 8-20% in unipolar depressed patients (Akiskal and Benazzi, 2003; Sato et al., 2004). Irritability has been found in 57% and depressive psychomotor agitation in 39% of bipolar depressions (Judd et al., 2012). Inter-episode stability of depressive mixed states (defined as 2 or more manic symptoms) was found in the Sato study to be 50% and in the study by Maj to be as high as 65% (Maj et al., 2003). A family history of bipolar disorder is far more common in both unipolar and bipolar patients exhibiting mixed depressive states (Akiskal and Benazzi, 2003; Benazzi, 2005; Ducrey et al., 2003; Maj et al., 2006; Sato et al., 2003a) and suggestions have been made to include all unipolar depressives exhibiting manic symptoms into the bipolar category (Akiskal and Benazzi, 2003). Of the manic like symptoms, irritability and psychomotor agitation appear as those having the greatest specificity and positive predictive value for describing a mixed depressive state (Benazzi and Akiskal, 2006). Depressive psychomotor agitation has been found to most commonly alternate with episodes of depressive psychomotor retardation in bipolar patients compared with those patients, often older, who have recurrent major depression where solely agitation recurs (Angst et al., 2009). Since the large scale introduction of antidepressants it has been noted that this group of patients respond poorly to antidepressants alone, often requiring antipsychotics or valproic acid to respond (Koukopoulos et al., 2007; Yatham et al., 2005) suggesting a different biological pathophysiology to unipolar depressions.

Yet in contrast to the above studies, Goldberg (2009b) found that when examining the numbers of manic symptoms in bipolar depression the percentage of patients with no manic symptoms was 31%, with one manic symptom 25%, with two 18%, with three 11% and with four or more 15%. This would suggest that mixed episodes are not discrete categories rather a spectrum of both poles of mania and depression operating on a continuum, relatively autonomously of one another and thus indicating different biological underpinnings. These findings have led to "mixed state" being deleted as a separate episode type from the proposed DSM 5 bipolar disorder section and instead it is suggested that manic or depressed episodes are specified as having mixed features if 3 or more symptoms of the opposite mood state are present, still leaving the predicament what to call the other approximately 45% (or 59% according to the earlier quoted studies) who have one or 2 symptoms of opposite polarity. Once again, mood incongruent psychotic symptoms have been left out of the examinations and thus the definitions.

1.2.3 The Effect of Temperament on Symptomatology During Mood Episodes

Temperament in this context is defined as an enduring aspect of one's personality that is thought to be innate rather than learned. With relevance to biological phenotypes of bipolar disorder, temperament is hypothesized to reflect the same biological/behavioural processes that drive the illness. In short a person may be described as **hyperthymic** when being full of energy, confident, self-reliant, an habitual short sleeper, novelty seeking, exuberant, versatile and with high libido or **depressive** (the opposite of the above) or **cyclothymic** where periods of the above two ways of being alternate in rapid succession often without external cues. Akiskal and colleagues (1998b) have identified a fourth type of temperament, that of **irritable** which is manifested by moodiness and being easily angered, the liberal use of sarcasm, a tendency to brood, impulsivity and dysphoric restlessness which alternate with infrequent periods of euthymia). Earlier, Kraepelin noted the importance of a person's dominant temperament on the clinical presentation of mania or depression (Akiskal, 2002) and that, whilst one polarity predisposed to the corresponding type of mood episode, it was possible for a person to experience the opposite polarity mood episode. The temperamental subtypes of cyclothymic, depressive and irritable have been found to be associated with mixed manic episodes (Akiskal et al., 1998a; Azorin et al., 2010; Rottig et al., 2007). Mixed episodes have been thought to be the result of the intrusion of the opposite temperamental pole to the episode: a depressive or irritable temperament intruding into mania or a hyperthymic temperament intruding into depression.

Clinically it has been long recognized that there is an overlap in emotional states between bipolar 2 disorder and borderline personality disorders (see review by Magill (2004)). Attempts have been made to delineate these conditions (Henry et al., 2001; Wilson et al., 2007) focussing on affective instability, hostility and impulsivity. However affective lability and affect intensity have been suggested to be core dimensions of bipolarity during euthymia even in bipolar 1 disorder (Henry et al., 2008) although in this study borderline personality disorder was not controlled for in the analysis. Several studies have examined neuroticism and extraversion in bipolar disorder finding evidence for increased neuroticism (Barnett et al., 2010; Jylhä et al., 2010) and reduced extraversion (Barnett et al., 2010). Yet there does not appear to be an effect of these factors on whether or not somebody manifests mixed affective episodes (Kim et al., 2011; Rottig et al., 2007). Other personality styles that have been associated with bipolar 1 disorder with mixed episodes are those in cluster C according to DSM – (ie) avoidant, dependent and obsessional (Rottig et al., 2007) though this cluster has in other studies been found to be more common in those with recurrent unipolar disorder than in the pooled bipolar disorder group (Brieger et al., 2003). Interestingly this cluster also manifests with greater frequency of lifetime anxiety disorders when the person has a bipolar disorder (Rottig et al., 2007).

1.2.4 Establishing Phenotypes for Genetic Analysis

Bipolar disorder is mostly a remitting disorder which implies that the biology of it must combine both state and trait factors. As seen above the symptoms are also highly heterogeneous and the clinical picture varies markedly between patients. Furthermore classifications differ and even the conceptualization of bipolar disorder and the cycloid psychoses remain controversial with little agreement on what to regard as core symptomatology outside of the classic euphoria/depression. The likelihood of finding a particular pathophysiology or gene or small group of genes wholly responsible for the disorder is thus unlikely even though heritability of the disorder is estimated at 80% (Shih et al., 2004). In fact, in common with

other complex disease conditions, single gene variations have not been found and data from genome wide association studies (GWAS) have identified genes with small contributions to the overall risk of developing the disorder (Schulze, 2010) leading researchers to ask “where is the missing heritability?” (Maher, 2008). As a result it has been proposed that refining the studied phenotypes is crucial in attempting to describe this heritability.

An approach to getting around the problem of noise in syndromes such as bipolar disorder not yet known to biologically equate to an underlying disease is to deconstruct the syndrome into its parts: symptoms, biological markers and pathways involved in biological systems of relevance (Schulze et al, 2005). Subsequent to this one can reconstruct a phenotype (reverse phenotyping) by using, for example, the genotype to observe what symptoms and signs are related to the particular genotype. The saga of the gene *G72* illustrates this approach well as polymorphisms in this gene have been identified to confer risk to schizophrenia, bipolar disorder, major depression and panic disorder whilst correlating in the general population to high neuroticism. This neuroticism factor is now thought to underlie the vulnerability to the above psychiatric diagnoses (Rietschel et al., 2008). Perhaps the genetic contribution to complex psychiatric disorders lies in a variety of genes contributing interactively across psychiatric syndromes and that current syndromic definitions will not at all help us in defining the underlying heritability.

So where does this leave us? Perhaps going back to basics and combining one or several symptoms with what is known about underlying biology of these symptoms may be a starting point (Schulze, 2010). An example of this approach is the association found between persecutory delusions in bipolar disorder and *DAOA/G30* (Schulze et al., 2005). A number of studies have examined symptoms and conditions associated with bipolar disorder that have been found to be familial with gene polymorphisms. For example, anxiety and neuroticism co-morbidity (Campos et al., 2010b), psychosis (Benedetti et al., 2010; Goes et al., 2007), suicide attempts (Campos et al., 2010a), age of onset and course (Smeraldi et al., 2002). However it is difficult to know whether these rather disparate genetic findings reflect contributions to a more generic “severity” process which may cut across a number of diagnoses or are specific to bipolar disorder.

1.3 Neurosteroids

1.3.1 Concepts

Since Baulieu and colleagues coined the term neurosteroid in 1981 (Baulieu, 1981) it has been clear that the picture of sex steroids, traditionally thought of as sex hormones acting solely as hormones at peripheral and removed sites from their production, was erroneous. The realization that these have major effects in the brain independent of sex is one of the quiet revolutions in medical discovery in the latter part of the 20th century, the repercussions of which have not yet been fully realized.

1.3.2 Biosynthetic Pathways

Traditionally it has been taught that oestrogen and testosterone are respectively female and male sex hormones which are produced in the ovaries and testes respectively after stimulation by luteinising hormone (LH). It has been said that stimulation of spermatogenesis and ovulation occurs, grossly simplified, with follicle stimulating hormone (FSH) in combination with LH. Significant progesterone synthesis in females has been said to occur in the luteal tissue during the luteal phase of the menstrual cycle. Adrenal production of sex steroids notably dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) have been

typically regarded as being the inactive metabolites on the road to conversion to oestrogen and testosterone. However, studies of a condition associated with a number of different steroid enzyme variations exhibiting disturbed patterns of steroidogenesis, congenital adrenal hyperplasia (CAH), have shown that for example DHEA has androgenic properties of its own. The majority of these CAH are caused by mutations in the genes coding for the enzyme 21-hydroxylase (Morel, 1997) although a number of other mutations in related genes are known to produce a similar phenotype (Krone and Arlt, 2009). Subsequently it has in women been found that adrenal production of testosterone equals that of the ovarian production (0.5-1.5nmol/liter) and requires *no* stimulation by LH (Piltonen et al., 2002). In males this same amount is produced in the adrenals, but the percentage produced by the testes is of course much higher (Diver, 2006). Progesterone is also manufactured at similar rates in the adrenals of men and women, without being controlled by LH or FSH (Piltonen et al., 2002). During the follicular phase of the menstrual cycle (days 1-14), adrenal synthesis of progesterone accounts for virtually all of the peripherally circulating serum progesterone. So do these compounds get from the periphery into the brain? Peripherally synthesised progesterone, testosterone (Pardridge and Mietus, 1979) and DHEAS (Asaba et al., 2000) all cross the blood brain barrier. In the case of DHEAS there is a positive gradient barrier whereby the brain concentrations in the human are 6-10 times that in plasma (Baulieu et al., 2001). Additionally, all these compounds have been either found to be synthesised in the brain or thought to be synthesised in those cases where the usual enzymes have not yet been clearly identified. Once within the brain they act locally, intimately connected with neurotransmission and other cellular pathways as well as with neural development and cellular migration the details of which will be explored below.

A number of enzymes are involved in steroidogenesis (Figure 2). These are by and large the same across all tissues though isoforms may differ. In this discussion, focus will be placed on the three enzymes that were chosen for our studies. Reference will be made to their roles in both humans and animals.

1.3.2.1 3β -Hydroxysteroid Dehydrogenase (*HSD3B1* or 2 also known as 3β HSD)

This enzyme is essential in a number of reactions in the steroidogenic pathway i) converting the precursor pregnenolone to progesterone, ii) converting 17-hydroxy-pregnenolone into 17-hydroxy-progesterone, thereby limiting the amount of 17-hydroxy-pregnenolone available for conversion into DHEA(S)¹, iii) converting DHEA into the more potent androgen androstenedione, iv) converting androstenediol into testosterone. There are two isoforms: HSD3B1 is found in placenta, breast and in thalamus, hypothalamus, hippocampus, olfactory bulb, caudate and nucleus accumbens in mammals as well as in Purkinje cells of the cerebellum (Do Rego et al., 2009). HSD3B2 is found predominantly in the adrenal gland and, at lower concentrations, in the brain especially amygdala, corpus callosum, hippocampus, caudate and thalamus (Yu et al., 2002). The enzymes are located in the endoplasmic reticulum as well in mitochondria (Simard et al., 2005) in astrocytes (Zwain and Yen, 1999b) as well as in hippocampal neurons (the latter in rats at least) (Higo et al., 2011). 17β -hydroxysteroid dehydrogenase which is active in other parts of steroidogenesis can supplement its activity (Suzuki et al., 2000).

Gamma-aminobutyric acid type A (GABA_A) receptor stimulation reduces enzyme activity in a local negative feedback loop (Do Rego et al., 2000). Furthermore artificial substances such

¹ DHEA(S) stands for DHEA in addition to DHEAS

as perfluoroalkylated substances also inhibit enzyme activity (Zhao et al., 2010). Perfluoroalkylated substances are widely used in industrial and consumer applications including stain-resistant coatings for fabrics and carpets, oil-resistant coatings for paper products approved for food contact, fire fighting foams, mining and oil well surfactants, floor polishes and insecticide formulations. Penetration into food substances has been suspected but the effects in humans remain uncertain (<http://www.efsa.europa.eu/en/data/call/datex100429.html>). Trilostane, an inhibitor of HSD3B2, has been found to have antidepressant like effect in mice by reducing immobility time in the forced swim test. The mechanism appears to involve decreased hippocampal progesterone, increased pregnenolone and increased serotonergic and norepinephrine turnover (Espallergues et al., 2009; Espallergues et al., 2012). However trilostane also has androgen receptor agonistic effects (Takizawa et al., 2010) which have not been accounted for in the above observations and may limit treatment in certain groups such as those at risk for prostate cancer.

The genes *HSD3B2* and *HSD3B1* are both located at 1p13.1 and are separated by 84kb (Shimodaira et al., 2010). Several gene polymorphisms in *HSD3B1* have been associated with serum aldosterone concentration differences and essential hypertension (Shimodaira et al., 2010). Somewhat tangentially rs10754396 in *HSD3B2* has been associated with differences in sex hormone binding globulin concentrations even when allelic differences in the *SHBG* gene were taken into account (Ahn et al., 2009). There have been no published investigations into links between these enzymes, their polymorphisms and psychiatric disorder.

Transcription of the *HSD3B1/2* genes is stimulated by ACTH, by NGFIB (nerve growth factor induced clone B - also known as NUR77 as well as NR4A1 and previously as NOT [(Bassett et al., 2004)]) and by NURR1, another member of the NGFIB family (Rainey and Nakamura, 2008). Furthermore transcription is regulated by cytokines (IL4), growth factors and prolactin via activation of tyrosine kinase and signal transducer and activators of transcription so called STAT's (Darnell, 1997). Testosterone, (Heggland et al., 1997) as well as oestrogen (Pradhan et al., 2010), have been found to decrease messenger ribonucleic acid (mRNA) of HSD3B in a negative feedback loop.

In conclusion, the enzyme is not only regulated by other sex hormones but also by the GABAergic system as well as a number of brain growth factors that have been separately shown to be of interest in psychiatric disorders.

1.3.2.2 Steroid-5-alpha-reductase (*SRD5A1*)

This enzyme converts progesterone into the intermediate product 5- α -dihydroprogesterone, as well as testosterone into dihydrotestosterone (DHT) which is a more potent androgen than testosterone, and additionally converts 11-deoxycorticosterone to 5 α -dihydrodeoxycorticosterone (5 α DHDOC). There are 2 isoforms – SRD5A1 which is expressed in human brain especially in cortex, subcortical white matter, cerebellum, hypothalamus and pons at all developmental phases (Do Rego et al., 2009) and SRD5A2 which is preferentially expressed in testis and appears to be lacking from adult human brain. In rats, it is expressed in late foetal and early neonatal period (corresponding to human foetal life) leading to speculation that it is involved in brain sex differentiation (Poletti et al., 1998a; Poletti et al., 1998b). The enzyme appears in neurons (for example, hippocampal pyramidal cells), oligodendrocytes and astrocytes, and is localized in the microsome. The type 1

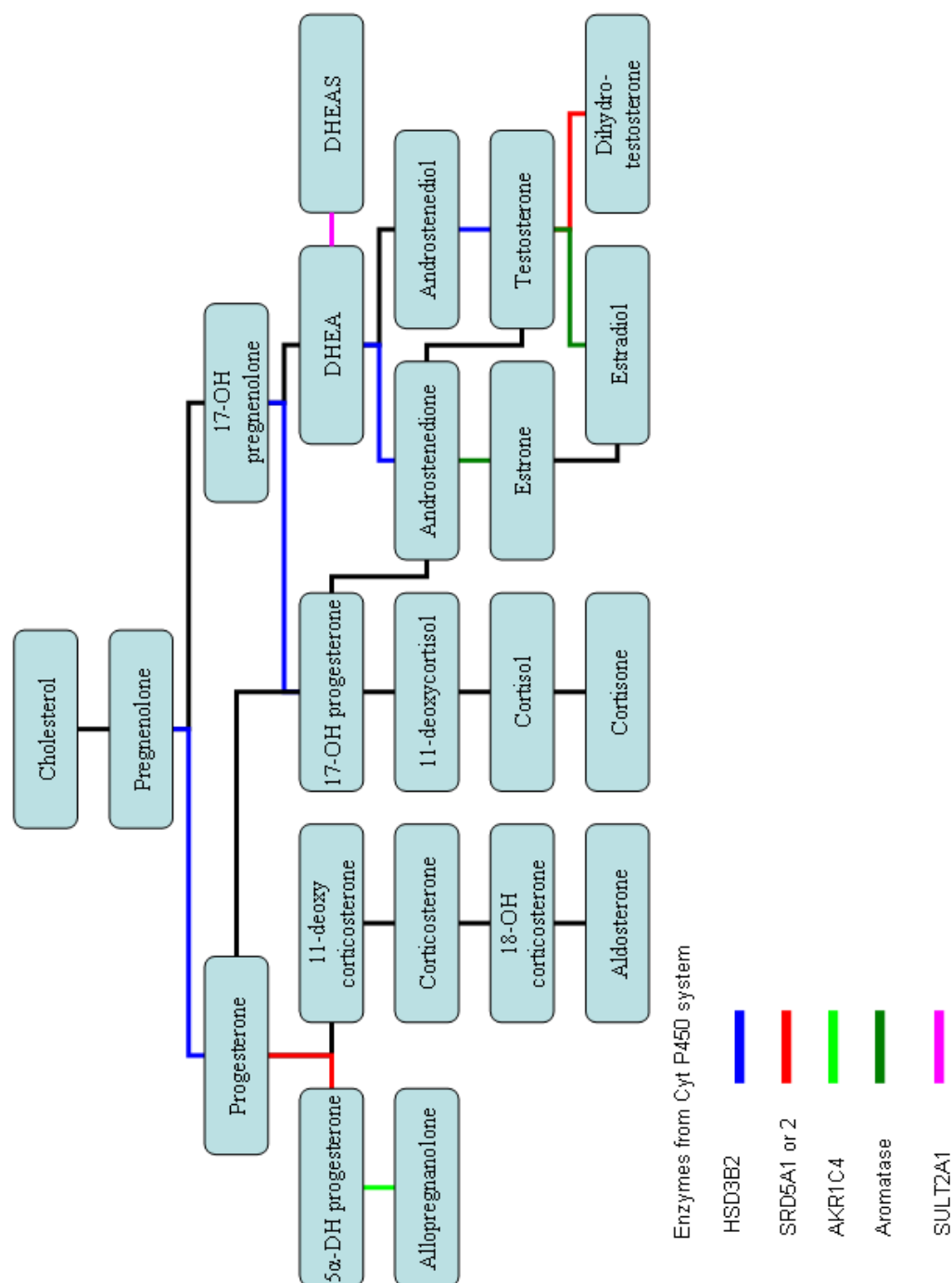


Figure 2: Steroidogenic pathways with involved enzymes

isoform is expressed equally between sexes (Melcangi et al., 1998) and at physiological pH favours progesterone over testosterone as a substrate (Poletti et al., 1998a) whereas the type 2 isoform favours testosterone

Testosterone and DHT increase gene expression of SRD5A1 even though these are not the primary substrates (Torres and Ortega, 2003). Nerve growth factor stimulates SRD5A1 mRNA (Her et al., 2004). Chronic placental hypoxia has in sheep been found to upregulate SRD5A1 (Nguyen et al., 2003). Prolactin has been found to reduce enzyme activity (Lobo and Kletzky, 1983).

Of relevance to psychiatry, SRD5A1 expression has been found to be decreased in CA3, basolateral amygdala, glutamatergic cell layers 5 and 6 frontal cortex but not in thalamus or striatum in socially isolated mice male mice. This was found regardless of whether treatment with anabolic steroids had been given (Agis-Balboa et al., 2007; Pinna et al., 2008). The reduced expression of SRD5A1 led to reduced allopregnanolone (ALLO) and was thought to be of significance for the development of fear related behaviours. Conversely, rearing in enriched environments increased SRD5A1 expression (Munetsuna et al., 2011). Taking this research track a step further, restraint stress as opposed to social isolation, has been found to give sexually dimorphic effects on SRD5A1. In males, expression of enzyme was increased in the prefrontal cortex and associated with increased local progesterone concentrations whereas in women expression was reduced but without altered local progesterone concentrations (Sanchez et al., 2009). Finasteride, which is used in treating androgenic alopecia, inhibits SRD5A2 more powerfully than SRD5A1 and has been found to cross the blood brain barrier (Russell and Wilson, 1994) abolishing stress-induced elevation of ALLO (Mukai et al., 2008) and the expression of fear behaviours in rats. Finasteride has also been suggested to be mildly antipsychotic in effect (Paba et al., 2011); it alters prepulse inhibition deficits in rat models of schizophrenia (Devoto et al., 2011). How this is done is not yet clear as direct dopaminergic effects have not been confirmed despite evidence of elevation of dopamine in the nucleus accumbens when downregulation of SRD5A occurred in socially isolated rats (Bortolato et al., 2011).

The 2 enzymes are coded for by separate genes not located on the same chromosome. *SRD5A1* gene is located on 5p15. Gene variants have been found to be associated with increased risk of polycystic ovarian syndrome (PCOS) and, in particular, with the degree of hirsutism, indicating that this enzyme presumably affects the production of additional androgens (Goodarzi et al., 2006). Other clinical association studies with polymorphisms in the *SRD5A1* gene have thus far largely focussed on testosterone/dihydrotestosterone activity and male patterned baldness, hypospadias, prostatic cancer or hypertrophy (Ellis et al., 2005; Ellis et al., 1998; Ha et al., 2003; Setlur et al., 2010; Tria et al., 2004), or female hyperandrogenism (Eminovic et al., 2005). Another study implicated gene variants as important in determining outcome in myeloma treatment (Van Ness et al., 2008), the mechanism of which is unknown. SRD5A1 polymorphisms have also been implicated in peripheral vascular disease, underlining the diversity of roles the biological substrates and products of this enzyme have in the body (Signorelli et al., 2008). One study found an association between several single nucleotide polymorphisms (SNP's) in this gene in bipolar disorder (Steen et al., 2010) but after correction for multiple testing this association disappeared. One study has found an increased risk for alcohol dependence (Milivojevic et al., 2011).

SRD5A2 is found at 2p23. Polymorphisms have been implicated in risk for sexual differentiation disorders (Fernandez-Cancio et al., 2011), prostatic hypertrophy (Izmirli et al., 2011), PCOS (Graupp et al., 2011), risk for early onset prostate cancer in Caucasians (Wang et al., 2010) and differences in sperm motility (Elzanaty et al., 2006). The only study thus far examining *SRD5A2* in psychiatric disorders found an increased risk for schizophrenia with polymorphism rs6732223 with evidence of increased enzyme activity (Steen et al., 2010).

1.3.2.3 Aldoketoreductase (AKR1C, also known as 3 α -Hydroxysteroid Dehydrogenase Type 1 as well as 3 α HSD as well as 3 α HOR)

This is one in the extremely large family of aldoketoreductases found in the body where the nomenclature has changed over time and between different research groups making the reading of the literature all the more difficult. Regular large reviews of subpopulations of this family do occur and it is on these reviews that the following picture is drawn. This enzyme converts 5- α -dihydroprogesterone into 3 α 5 α -progesterone (ALLO), dihydrotestosterone into 3 α -androstenediol and dihydrodeoxycorticosterone (DHDOC) into tetrahydrodeoxycorticosterone (THDOC). *AKR1C* occurs in a number of isoforms in humans but not in rats where only one isoform exists (Do Rego et al., 2009). In mice and rats at least, *AKR1C* enzyme and *SRD5A* are co-expressed in the same cells in the CNS but not in the GABAergic interneurons, rather in these interneurons output neurons (Agis-Balboa et al., 2006).

In humans type 1 (*AKR1C4*) is not expressed in brain but is extremely common in liver and is involved in peripheral synthesis and metabolism of progesterone along with other steroids. Type 2 (*AKR1C3*) is found in prostate, mammary glands, temporal lobes, putamen, medulla, spinal cord and in subcortical matter. Type 3 (*AKR1C2*, aka 17-hydroxysteroid dehydrogenase type 10) is found preferentially in amygdala, hippocampus and hypothalamus (He et al., 2005) but also in putamen, cerebellum, medulla and spinal cord (Do Rego et al., 2009). This enzyme inactivates oestradiol, synthesizes dihydrotestosterone and converts allopregnanolone back to 5 α -dihydroprogesterone (Hovorkova et al., 2008). The activity in the brain appears to be one tenth of that in liver (Steckelbroeck et al., 2001). Type 4 isoform known as 20 α (3 α)-HSD (*AKR1C1*) is also found in the brain in a wide distribution (Penning et al., 2000). The enzymes are all found in the mitochondria. Some isoforms can supplement the role of *HSD3B2* by working in the opposite direction of the usual conversion reactions in the steroidogenic pathway (Steckelbroeck et al., 2004a). This appears especially so for *AKR1C4* which is between 10-30 times more efficient than the other isoforms in converting substances (Penning et al., 2000; Penning et al., 2003). The directionality of conversions is governed by the redox-state of the cell, which changes as a result of, for example, oxidative stress (Penning et al., 2004).

Rearing rats in enriched environments markedly increased *AKR1C* expression (Munetsuna et al., 2011). Interestingly, a greater than normal left hippocampal asymmetry of expression of this enzyme has been found in Alzheimer's disease and in schizophrenia (Hovorkova et al., 2008).

The genes coding for the above enzymes are all located at 10p15-p14 but with some distance between them. Polymorphisms in *AKR1C4* have been implicated in a range of disorders for example, risk of prostate cancer in the presence of oestrogenic insecticides (Multigner et al., 2010), mammographic density changes with use of combination oral contraceptive/ replacement therapy (Lord et al., 2005), reduced risk of breast cancer during oestrogen monotherapy (Hein et al., 2011), differences in nicotine metabolism and risk of lung cancer (Ter-Minassian et al., 2011). *AKR1C3* polymorphisms have been associated with childhood leukaemia (Liu

et al., 2008a), potentially conferring added risk for carcinogen mediated leukaemia (Birtwistle et al., 2009) and bladder cancer (Figuerola et al., 2008).

In summary, the biosynthesis of neuroactive steroids has been found to occur at multiple sites in the body and in many cases also in the brain. Surprisingly, in the brain there appear to be few sex differences in the localization or concentration of these enzymes. Stress has been shown in some cases at least, to affect these systems in sex specific ways. The pathways and directionality of the chemical reactions are controlled by a wide range of substances that are themselves not directly involved in the biosynthetic pathways. These include oxidative stress, growth factors in the brain as well as exogenous substances. Polymorphisms of the genes coding for these enzymes have not yet been extensively characterized yet when they have they have been examined they have been associated with a wide range of health problems highlighting the manifold processes the steroids are involved with.

Having discussed the enzymes and the genes coding for these it is now time to look more closely at some of the products of these conversions and their roles in the brain. Once again the focus will be on the sex steroids selected for study but as there are overlapping effects between DHEAS and its precursor DHEA these two will be considered together.

1.3.3 DHEA and DHEAS

1.3.3.1 Factors Influencing Synthesis and Metabolism

Adrenal Synthesis:

Developmentally, in humans but not in rats (Wolf and Kirschbaum, 1999), DHEA and DHEAS are produced in the inner foetal zone from week 8 of gestation and production peaks around delivery after which there are very low circulating levels of these two substances until adrenarche when the adrenals once again manufacture these, especially DHEAS, in large quantities. Production peaks in the mid 20's to thereafter fall by ca 1-1.5% per year. In adulthood DHEA(S) are synthesized in the zona reticularis corresponding to the inner foetal zone (Wolf and Kirschbaum, 1999). There is a pronounced diurnal variation (Rosenfeld et al., 1975) with lowest levels in the afternoon and a pulsatile pattern more or less synchronous with cortisol.

The age related variations in synthesis are accompanied by differences in gene expression of the enzymes of steroidogenesis in the adrenal gland as well as differential expression during development of growth factors and transcription regulators including NGFIB and NURR1 (Ishimoto and Jaffe, 2011). These temporal variations, which mirror those of important brain developmental periods and those of several neurodevelopmental psychiatric disorders such as schizophrenia and bipolar disorder, make DHEA and DHEAS attractive pathophysiological candidates in these disorders.

Brain Synthesis and Metabolism:

Animal experiments in the early 1980s showed that pregnenolone and DHEA concentrations in the brain were not reduced by adrenalectomy and castration (Corpechot et al., 1981) indicating that there was de novo synthesis in the brain. Despite this the concentration of DHEA in the cerebrospinal fluid (CSF) correlates however well with that in serum (Kancheva et al., 2011). DHEA is predominantly synthesised in astrocytes and oligodendrocytes but also in neurons including pyramidal cells (Kimoto et al., 2001; Zwain and Yen, 1999b). Although it was also deduced from extraction experiments from 1981 onward that sulphated esters of DHEA existed in the rat brain (Corpechot et al., 1981) it has been recently recognized that DHEAS does not appear to be nearly abundant as thought in

the rat brain (Liere et al., 2004). The reason for this appears to be that previous extraction technology failed to distinguish DHEAS from the lipoidal form of DHEA. It is thus unclear whether the numerous experiments conducted in rats using infusions of nanomolar concentrations of DHEAS reflect the *in vivo* situation where concentrations are below this range. *In contrast to rats, the human brain continues to show high concentrations of DHEAS with the new methodology* (Lanthier and Patwardhan, 1986; Steckelbroeck et al., 2004b). The situation in humans is also more complex than first thought as concentrations of DHEAS in the brain are between 6 and 10 times those of plasma yet the classical sulpho-transferase enzyme has not been identified (Steckelbroeck et al., 2004b) leading to the speculation that DHEAS is selectively transported into the cells via recently identified transport proteins (Fang et al., 2010; Kullak-Ublick et al., 1998) both across cell types and across the blood brain barrier. Another possible mechanism for *de novo* brain synthesis may be derivation by other as yet unknown pathways. Thus far the working hypothesis has been that DHEAS, despite the concentration differential, is mainly transported into the brain and there acts as a neuroactive steroid whereas DHEA is manufactured in the brain largely from the removal of the sulphate moiety of DHEAS.

The relative synthesis and metabolism of DHEA compared with other neurosteroids appears to be coordinated regionally between cell types with the exchange of intermediaries across cell membranes, a process utilizing transport proteins (Zwain and Yen, 1999a). Regional differences in metabolism occur because of differences in enzyme expression, for example, the metabolite androstenediol is more commonly made in cerebellum while 7 α -hydroxy DHEA is made in the frontal cortex (Weill-Engerer et al., 2003). The significance of this is still largely unknown. Interestingly, the directionality of metabolism may be influenced by cell density (Akwa et al., 1993), the mechanism of which is still unknown.

Factors Affecting Metabolism:

Genetic factors as shown in twin and family studies are thought to account for between 40-65% of the variability in serum DHEAS concentrations controlling, for example, the degree to which severe exercise changes DHEAS concentrations (Riechman et al., 2004). A finding without as yet clear mechanistic explanation is that vitamin D receptor polymorphisms appear to correlate with the amount of DHEA synthesized in later life (Zofkova et al., 2002a). Still other polymorphisms in CYP3A7 affect how much DHEAS is produced in adulthood and can decrease circulating DHEAS by 50% (Smit et al., 2005). The apo E4 genotype associated with Alzheimer's disease has been associated with increases in serum DHEAS however the mechanism for this is unclear, possibly occurring via cholesterol transport pathways (Zofkova et al., 2002b). However, the suggestion has been made with the discovery of lipoidal DHEA present in cell membranes, that this type of DHEA may in some way interact with extracellular DHEA/DHEAS altering concentrations and that this could potentially be the mechanism behind the Apo E genotype differences (Liere et al., 2004). Free radicals have been shown to stimulate production/presence of DHEA(S)² within the brain (Maayan et al., 2005b).

Medications such as clozapine (Nechmad et al., 2003) and lithium reduce rat frontal cortex and hippocampal DHEAS and DHEA without influencing peripheral concentrations via the

² where DHEAS is mentioned with regards to rats or mice it refers to this being given in variable doses in animal experiments; with the earlier finding of lipoidal DHEA being the vast majority of the "DHEAS" previously found in rats and mice it is difficult to interpret the physiological relevance of this in just rats and mice. However as DHEAS is abundant in human brain, the experiments may still give valuable insights into how DHEAS affects humans.

inhibition of phospho-adenosine phosphates (PAP) (Maayan et al., 2004). Other medications such as methylphenidate may increase DHEAS, at least in boys (Maayan et al., 2003) whereas mirtazapine has been shown to be associated with a reduction in DHEAS over the course of treatment although it is difficult to say whether this is a specific effect of the medication or an association with improvement in depression (Schule et al., 2009). Interestingly neither low to moderate alcohol ingestion (Dorgan et al., 1994), nor naltrexone (Ceballos et al., 2007), smoking (Soldin et al., 2011) or olanzapine (Bicikova et al., 2011) affect serum DHEAS concentrations. Valproate normalizes DHEAS in women long-term but relative to lithium increases it (McIntyre et al., 2003). The acute effect of valproate in females is to either reduce DHEAS or leave it unchanged (Rattya et al., 2001a) whilst it has been suggested to increase DHEAS chronically in males (Rattya et al., 2001b). Carbamazepine reduces DHEAS in both sexes (Rattya et al., 2001a).

1.3.3.2 *Role in Neurogenesis and Neuronal Survival*

DHEA has been found to increase spine density in rat neuronal axons and to promote neurogenesis (review by Charalampopoulos 2008) in the mouse both developmentally and after injury (Fiore et al., 2004). There appear to be dose dependent window of effect as only moderate to high normal doses have beneficial effect (Marx et al., 2000) and not low concentrations (Marx et al., 2000) or supraphysiological doses (Li et al., 2009; Patel and Katyare, 2006). The mechanisms for the neurogenesis appear numerous including stimulation of various nerve growth factors (Gubba et al., 2004), Protein kinase 3 (Kimonides et al., 1999), Bcl-2 proteins (Charalampopoulos et al., 2004) and direct inhibition of NO synthase downstream of N-Methyl-D-aspartate (NMDA) receptor activation (Kimonides et al., 1998; Kurata et al., 2004) as well as enhanced scavenging of free radicals (Katyare et al., 2006; Patel and Katyare, 2007). The antioxidant effect was one of the earliest documented (Araghiniknam et al., 1996; Aragno et al., 1997; Bastianetto et al., 1999; Boccuzzi et al., 1997). As a consequence of the above and other neuroprotective findings, dietary supplements or skin creams of DHEA have been viewed as the “elixir of youth” – potentially counteracting aging effects in skin (Tagliaferro et al., 1986) and of the brain. These have been marketed in the US as a food supplement evading the strict control system of the Food and Drug Administration. As a consequence of this, DHEA has been readily available for clinical trials (see below).

By contrast DHEAS increases apoptosis during development (Zhang et al., 2002) suggesting that the balance of DHEA and DHEAS may be vital in correct synaptic and cellular pruning during early life. Abnormalities in cellular pruning have been seen both in schizophrenia and in bipolar disorder (Garey, 2010; Rajkowska et al., 2001; Selemon et al., 1998) the underpinnings of which have not yet been fully elucidated (Connor et al., 2010). However, in developed neural cells both DHEA and DHEAS appear to be neuroprotective (Bologa et al., 1987). DHEAS also increases neurite length via marker microtubule-associated protein-2 (MAP2) (Compagnone and Mellon, 1998). Increased MAP2 staining neurons have been found for example in some brain regions in schizophrenia and bipolar disorder (Baxter et al., 2006; Bouras et al., 2001; Connor et al., 2010).

1.3.3.3 *Effects on Neurotransmission and the Catecholamine System*

Both DHEA and DHEAS act by intracellular binding of the sigma 1 receptor ($\sigma 1$) (Bergeron et al., 1996; Su et al., 1988), which more correctly can be thought of as an intracellular protein as opposed to a receptor. The $\sigma 1$ receptor will be explored in greater detail below. In binding with the $\sigma 1$ receptor DHEAS(S) allosterically *activates* the NMDA receptor

(Johansson et al., 2005). The effects at this protein are antagonized by allopregnanolone (see section below on allopregnanolone). Additionally, both DHEA (Imamura and Prasad, 1998; Majewska, 1992) and DHEAS (Sullivan and Moenter, 2003) act as negative allosteric modulators of the GABA_A receptor (Majewska et al., 1990) reducing GABA activation by other signals, see Figure 3. DHEA(S) exhibit therapeutic window effects such that very low concentrations allow activation of the GABA_A receptor and higher doses inhibit it (Majewska, 1992).

In short DHEA(S) can be viewed as having excitatory profiles in the central nervous and autonomic nervous systems. Yet this effect may be dose dependent given that the effects on both the NMDA receptor and the GABA_A receptor have been found to be bell shaped, i.e. optimal excitatory effect occurs at midlevel doses whereas low and very high doses are inhibitory (see section on sigma 1 receptor agonists 1.3.3.4).

1.3.3.4 Sigma Receptor Effects

The $\sigma 1$ protein shares no homology with other known proteins. It sits on the interface between the endoplasmic reticulum (ER) membrane (Hanner et al., 1996) and mitochondria (Hayashi and Su, 2007) where it ensures optimal calcium signalling from the ER to the mitochondria, essential in energy metabolism and here it is also involved in activation of antioxidant responses (Pal et al., 2012). From this interface once bound and activated it can translocate to the plasma or nuclear membranes (Hayashi et al., 2000) where it binds to the NMDA subunit of the glutamate receptor. Once at the membrane it occurs pre and postsynaptically and is especially concentrated in hypothalamus, pyramidal cells of the hippocampus, cortex, striatum, midbrain and dorsal horn of the spinal cord in rats (Alonso et al., 2000) and in humans (Sakata et al., 2007). It is known to modulate neurotransmission as opposed to facilitating gradient based uptake of ions in cells (Aydar et al., 2002; Cheng et al., 2008; Monnet et al., 1990). Apart from its modulation of the NMDA receptor, it interacts with Dopamine 1 (D1) receptors facilitating glutamate *release* in these neurons (Dong et al., 2007). D1 receptor bearing neurons are important in mediating plasticity and mediating spatial, incentive as well as extinction learning (El-Ghundi et al., 2007). The glutamatergic stimulation also enhances postsynaptic norepinephrine release (Monnet et al., 1996) Yet sigma-1 receptor agonists also appear to inhibit glutamate release at cortical nerve endings potentially being the mechanism behind reduced neuronal death associated with glutamate toxicity (Lu et al., 2012) implying there may be either dose or regional differences in the actions of the $\sigma 1$ -receptor.

Cocaine (Xu et al., 2010) and methamphetamine (Nguyen et al., 2005a) have been shown to exert their effects on locomotion via $\sigma 1$ receptor agonism. Interestingly many antidepressants including venlafaxine, fluvoxamine, fluoxetine, citalopram, and imipramine act as sigma receptor agonists at doses much lower than those required for serotonergic effect (Bermack and Debonnel, 2005; Dhir and Kulkarni, 2007). More selective $\sigma 1$ receptor agonists have therefore been investigated for antidepressant effect with promising results in animal models (Fishback et al., 2010) although so far the anxiolytic effect appear to outweigh the antidepressant effects. This anxiolytic effect appears not be accompanied by weight gain or sedation (Volz and Stoll, 2004) which may make these ligands attractive for drug development. Yet once again dose windows may exist as high dosing of an agonist has shown *anxiogenic* effects (Navarro et al., 2012). Sigma 1 receptor antagonists include neuroleptics like haloperidol (Fletcher et al., 1994) and trifluoroperazine (Coughenour and Barr, 2001) that have $\sigma 1$ receptor blocking effects at the same dosages that give dopamine

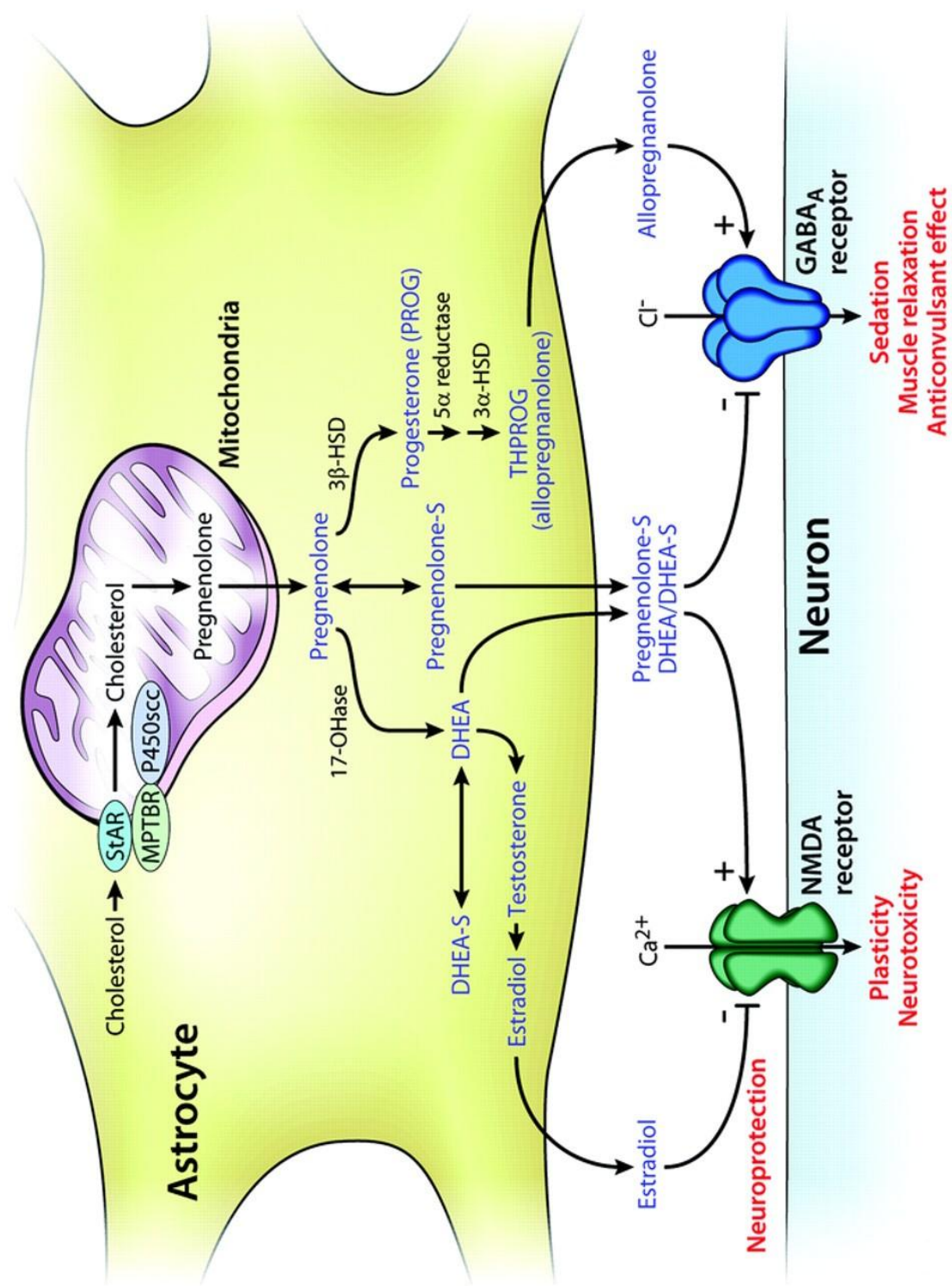


Figure 3: Actions of neurosteroids on NMDA and GABA receptors (used with permission from the Mayo Clinic)

receptor blockade (Cobos et al., 2008).

Sigma 1-receptor gene (*SIGMAR1*) variants have, in a meta-analysis of published studies, been found to increase risk of schizophrenia and were correlated with lower activation of prefrontal cortex in a subset of these sampled patients (Ohi et al., 2011). Variants have been also found to confer higher risk to developing Alzheimer's disease (Feher et al., 2012).

1.3.3.5 Other Effects in the Brain

DHEAS has been shown to synchronize hippocampal activity to theta rhythm (Steffensen, 1995) which has been shown to be essential in creating time and place maps in 3D space (Buzsaki, 2006). The mechanism for this effect appears to be muscarinic receptor agonism (Steffensen et al., 2006) in certain GABAergic interneuron cells which co-express muscarinic receptors. The same window effect is once again seen with dosing (Diamond, 2004; Diamond et al., 1996) and there appear to be interactive effects with psychological stress (Diamond et al., 1999).

1.3.3.6 Findings Related to Psychiatric Disorders and Behaviour

Cognition:

In terms of cognition in aging as well as potential associations with dementia, DHEAS in blood (Nasman et al., 1991) and in the brain (Weill-Engerer et al., 2002) has been noted to be reduced in severe Alzheimer's disease correlating with the presence of phosphorylated tau proteins in the hypothalamus. The relative ratio of DHEAS to DHEA in CSF separated out Alzheimer's disease from those with vascular dementia (Kim et al., 2003). These differences are in line with the reduced $\sigma 1$ ligand binding in PET studies on Alzheimer's disease (Toyohara et al., 2009). In an unselected population based study of the over 65's serum DHEAS has been found to correlate positively to mini mental state examination (MMSE) score and a lower concentration of DHEAS predicted also the greatest decline in MMSE over 3 years (Valenti et al., 2009). Administration of DHEAS improved behaviour and cognition in mice who had a mild traumatic brain injury induced (Milman et al., 2008) yet in humans improvements in cognitive functioning after brain injury or in dementia has by and large not been seen with DHEA supplementation even though serum DHEA(S) rise during treatment (Kritz-Silverstein et al., 2008; van Niekerk et al., 2001; Wolf et al., 1997). Some studies have documented a small effect of supplementation (Yamada et al., 2010) yet negative effects have also been seen (Parsons et al., 2006). Some of the reasons for these inconsistencies and lack of efficacy relate to the short half-life of DHEA (less than one hour) once DHEA is in the blood compartment (Wang et al., 1967). DHEA is also extensively metabolized in peripheral fat cells into other sex steroids (Luu-The and Labrie, 2010) which of themselves have differing effects on cognition (Hirshman et al., 2004).

Schizophrenia:

Cross sectional studies have found that low morning serum DHEA was associated with chronic schizophrenia but not with an acute schizophrenia episode (Tourney and Hatfield, 1972) while most studies agree that DHEAS is lower in male schizophrenia patients (Bicikova et al., 2011; Brophy et al., 1983; Garner et al., 2011) when emotional distress was controlled for (Ritsner et al., 2006). Only one study found no difference in DHEAS concentrations (Shirayama et al., 2002). However in the latter study there was a wide morning sampling period and age was not fully taken into account which may explain the negative finding. Another study found high concentrations of DHEA in the residual phase of schizophrenia (di Michele et al., 2005). Afternoon sampling has yielded no differences

between patients and controls in regard to DHEA (Gallagher et al., 2007; Tournay and Erb, 1979). In women with schizophrenia, DHEA has been shown to be elevated (di Michele et al., 2005). All the studies have been of small numbers and methods of analysis of DHEA(S) (whether radioimmunoassays or immunohistochemical methods were used) varied between studies which may influence concentrations obtained. In attempting to correlate particular symptom domains to DHEAS concentrations a positive correlation was found with cognition (Ritsner and Strous, 2010), particularly verbal memory and facial recognition (Silver et al., 2005).

As early as 1952 an open label clinical trial was conducted in patients with treatment resistant psychoses giving patients variable dosages of dehydroisoandrosterone (the older name for DHEA) which documented improvements in a range of functional domains within 1-2 weeks. These improvements reversed once the injections of medication were stopped (Strauss et al., 1952). Later a double blind placebo controlled trial with DHEA augmentation in men and a few women with schizophrenia gave positive effect on negative symptoms, however men and women were not analysed separately (Strous et al., 2007). Additionally, reduced prevalence of Parkinsonism has been seen with neuroleptics when patients also have been treated with DHEA (Nachshoni et al., 2005).

Mood disorders:

Given the sigma receptor activation effects of antidepressants and the clinical observation that DHEA administration in humans can trigger mania (Dean, 2000; Vacheron-Trystram et al., 2002) a number of studies have examined DHEA(S) in animal models of mood disorders as well as in humans with depression. Via σ_1 activation DHEAS acts to reduce fear conditioned immobility in rats and mice (Dhir and Kulkarni, 2008; Noda et al., 2000), one commonly used model for the learned helplessness paradigm of depression. Lower morning serum DHEAS was found in depressed compared with non-depressed women with Huntington's disease (Markianos et al., 2007a) and in both males and females with dysthymic disorder (Markianos et al., 2007b) yet other studies have found raised DHEAS pre-treatment in depression (Ozsoy et al., 2008; Takebayashi et al., 1998) and in menopausal women with depressive symptoms but without a clinical diagnosis of depression (Morrison et al., 2011). No difference was found in DHEAS concentrations in postmenopausal women with or without depression (Erdencler et al., 2004). Another study found no correlations with depression scores in unipolar depressed patients but did find a positive correlation with anxiety subscale scores (Hsiao, 2006b).

Given the findings of disturbances in the HPA axis in many persons with depression and post traumatic stress disorder (PTSD), DHEA has been analysed both on its own and as a ratio to cortisol, the hypothesis being that DHEA should rise to compensate for cortisol effects and if it does not then there is a greater risk for cortisol induced neurotoxicity (Ritsner et al., 2004). Morning DHEA has in males with posttraumatic stress disorder been found to be either lower (Olff et al., 2006) or higher than controls (Yehuda et al., 2006) and higher concentrations have been related to the degree of clinical improvement since worst symptomatic period of PTSD (Yehuda et al., 2006). In depression the cortisol:DHEA ratio has been suggested to be raised in the evenings but not in the morning (Young et al., 2002) and also that DHEA *reduces* as the depression lifts in those who remit (Fabian et al., 2001; Hsiao, 2006a). In at risk adolescents high salivary DHEA concentrations interacted with high scores on mood and feelings questionnaire to further increase the risk of developing clinical depression during the following year (Goodyer et al., 2000). In female patients with a diagnosis of borderline personality disorder, which by many is conceptualized as a form of early complex post

traumatic stress disorder, morning salivary DHEA was found to be higher especially in those that also met formal criteria for PTSD (Jogems-Kosterman et al., 2007). Furthermore, women with alcohol dependence who were undergoing controlled alcohol withdrawal treatment have been found to have elevated DHEA concentrations (Valimaki et al., 1990).

Aggression:

Animal experiments have shown that selective $\sigma 1$ activation reduced isolation induced aggression in rats and increased exploratory behaviour (Beltran et al., 2006). However DHEAS has also been shown to increase aggressive behaviour in male mice (Nicolas et al., 2001) and to be associated with higher aggression in monkeys (Goncharova et al., 2010), suggesting either species, context specific or dose specific differences. In humans, aggression was associated with higher DHEAS in conduct disordered male adolescents (van Goozen et al., 1998) yet lower DHEAS was found in males with first episode schizophrenia who had previously been aggressive (Strous et al., 2004) and in those who had previously been aggressive when examining DHEAS during early phase of alcohol withdrawal treatment. The latter showed a relationship with reduced suppression on dexamethasone suppression test but not with basal cortisol (Ozsoy and Esel, 2008). These results are perhaps not so contradictory if one remembers that there exist therapeutic windows of optimal dosing and that both extremes of concentrations are likely to give similar effects.

Stress:

In normal populations, higher DHEA has been found to correlate with less dissociation and higher performance on stressful underwater navigation training and testing in military personnel (Morgan et al., 2009) as well as to enhanced memory function via the activation of the hippocampus and anterior cingulate (Alhaj et al., 2006). Augmented DHEA response may even have a more direct survival value in reacting swiftly to danger as is demonstrated by reduced latency in P300 evoked response potential (Braverman et al., 2009). In contrast to this many chronic illnesses (Maninger et al., 2009) and general stress in healthy carers (Jeckel et al., 2010) associate with lower DHEAS concentrations. This is not the case with acute stress (Maninger et al., 2010). If serum DHEAS concentrations are generally lower in a range of chronic diseases can DHEAS predict prognosis? Survival in critical illness was associated with higher DHEAS in men but not in women (Sharshar et al., 2011) and along the same lines low serum DHEAS was associated with increased cardiovascular mortality in men (Ohlsson et al., 2010). It is not known whether this association also holds true within psychiatric populations who have increased risks for cardiovascular ill health (De Hert et al., 2009; Weiner et al., 2011). Higher DHEAS at baseline did indeed indicate better treatment response and continued abstinence from cocaine in one study of cocaine abusers (Wilkins et al., 2005) and DHEAS rose in depressed males who responded to electroconvulsive therapy (ECT) (Ozsoy et al., 2008). Yet starting out with a high DHEAS may not be advantageous in this setting: another study found that in the context of schizophrenia those persons whose depressive symptoms did not respond to ECT had high initial DHEAS (Maayan et al., 2000). Similarly in rats, ECT increased DHEAS but if ECT was given along with DHEAS then the antidepressive effect of reduced immobility in a learned helplessness paradigm was reversed (Maayan et al., 2005a). This suggests the importance of considering baseline values in considering the potential effectiveness of DHEA(S) augmentation in adjunctive therapy and the need to better understand the therapeutic window of $\sigma 1$ -agonists.

In summary, DHEAS tends to be lower in chronic conditions such as schizophrenia where it is associated with deficit symptoms. It increases in those who are treatment responsive, at least in depression. In contrast DHEA reactively increases when stress in many forms is

experienced, enhancing neuronal survival and contributing to the excitatory tone in the central nervous system. One can postulate that if there are conditions that prevent this reactive increase from happening, for example with genetic polymorphisms or differences in protein and gene expression, then oxidative stress and cell death may follow, not just from glucocorticoid neurotoxicity. DHEA(S) appears to act within a therapeutic window making it likely that both supraphysiological doses and very low normal doses are without effect. Additionally, a number of medications used as antidepressants and neuroleptics have sigma receptor effects at therapeutic doses and research is ongoing to find new σ_1 receptor ligands in the treatment of depression and anxiety. Findings also suggest σ_1 receptors are involved in the pathophysiology of Alzheimer's disease.

1.3.4 Progesterone and Allopregnanolone

1.3.4.1 Factors Affecting Synthesis and Metabolism of Progesterone and Allopregnanolone (Allo)

Both progesterone and ALLO are synthesized in the brain (Jung-Testas et al., 1989; Melcangi et al., 1994) in addition to crossing over the blood-brain barrier into the brain (Raisinghani et al., 1968). In brief, the cholesterol side chain is removed by P450_{scc} at the inner mitochondrial membrane to form pregnenolone. In the endoplasmic reticulum pregnenolone is converted by HSD3B2 enzyme to progesterone. The rate limiting step is thought to be the rate at which astrocytic cholesterol is transported from the outer to the inner membrane by steroid acute regulatory protein (StAR).

A number of psychiatric medications have been found to increase progesterone and allopregnanolone in the brain of rats. These include the antidepressants fluoxetine (Pinna et al., 2003), amitriptyline, desipramine (Jaworska-Feil et al., 2000) and mirtazapine (Pisu and Serra, 2004), the mood stabilizer carbamazepine (Jaworska-Feil et al., 2000), the antipsychotics clozapine (Barbaccia et al., 2001; Marx et al., 2003) and olanzapine (Marx et al., 2003). Interestingly, the antipsychotics haloperidol, sulpiride or risperidone did not affect concentrations (Barbaccia et al., 2001). In the case of the antidepressants, the effects on progesterone or allopregnanolone were obtained at doses that were 10% of those required for serotonergic or adrenergic effects (Pinna et al., 2003). There were variations between compounds between acute effects and chronic effects. For example fluoxetine's acute enhancement of ALLO normalized after several weeks of treatment whereas mirtazapine continued to show effects (Barbaccia et al., 2001; Serra et al., 2002). This was also shown in whether stress induced elevations of brain progesterone and ALLO: mirtazapine and imipramine abated the stress response (both with regard to corticosterone rises and catecholamine response (Dazzi et al., 2001a; Dazzi et al., 2001b)) and provided more continual anxiolytic effect whilst fluoxetine showed a more normal stress response allowing short term rises in anxiety (Pisu and Serra, 2004). Another interesting observation was that fluoxetine only raised brain allopregnanolone in rats raised in social isolation not in those who had been group housed (Pinna et al., 2006) showing the interaction between early environmental trauma, biological system differences in self-soothing and responsiveness to medications. As yet there have been no studies examining brain concentrations post-mortem in humans on and off antidepressants in an attempt to see if similar differences are seen in humans.

The only studies of medication effects in humans on serum concentrations of progesterone and ALLO have shown that 600mg clozapine did not raise serum allopregnanolone (Monteleone et al., 2004). This is in contrast to rats where the equivalent dosages of mg/kg

clozapine raised both brain and serum progesterone and allopregnanolone. The mechanisms behind these effects are thought to be i) induction of AKR1C enzyme in the brain (Jaworska-Feil et al., 2000), and ii) through the inhibition of the conversion of progesterone and allopregnanolone to their inactive metabolites. Particularly Cyp2D6 is important in this regard (Niwa et al., 2008). Although mirtazapine is not regarded traditionally as being metabolized by Cyp2D6 (Läkemedelsindustriföreningens Service AB, 2011) a number of publications have shown significant metabolism via this pathway (Borobia et al., 2009; Desmarais and Looper, 2009; Lind et al., 2009; Ramaekers et al., 2011). Given that Cyp2D6 is widespread in neurons and impacts a number of biological processes aside from drug metabolism (Bromek et al., 2010; Snider et al., 2008; Zhu, 2008) it is not surprising that it also affects neurosteroid metabolism.

1.3.4.2 Effects in the Brain by Progesterone

The male brain is more sensitive to progesterone than the female brain as shown, for example, by the extent of increase in EEG power when locally administered (Fernandez-Guasti et al., 2003). Progesterone also produces sexually dimorphic effects. For example, it increases inter-parietal lobe connectivity in males but reduces it in females (Fernandez-Guasti et al., 2003). These effects are mediated via i) the progesterone receptor which will be discussed in more detail below, as well as by ii) direct action on intracellular signalling cascades. Specifically, i) the mitogen-activated protein kinase (MAPK) pathway, a major signalling pathway involved in transcription and neuronal plasticity, function and survival, and ii) the Akt (Protein kinase B) cascade (Singh, 2001) which serves to regulate dopamine and serotonergic transmission (Beaulieu, 2011) as well as metabolism and apoptosis. The MAPK pathway has been implicated in schizophrenia (Funk et al., 2012) and as a shared site of action for mood stabilizers as molecularly divergent as lithium and valproate (Gupta et al., 2011) and clozapine (Pereira et al., 2009). Progesterone, via the Akt cascade and its effect on inositol 1,4,5-triphosphate (IP₃) receptors, increases the gain in calcium signalling making the cell more responsive to low amplitude stimuli (Hwang et al., 2009; Koulen et al., 2008) rather than higher amplitude stimuli, thus helping in fine tuning circuitry. Furthermore rapid modulation of calmodulin dependent protein kinase II and protein kinase C have been observed with progesterone (Balasubramanian et al., 2008a, b) both pathways being implicated in for example schizophrenia and depression (Novak et al., 2006) as well as being sites of lithium action (Sasaki et al., 2006). There may be regional differences in effects on these signalling cascades as well as differences between short and long-term effects of certain concentrations of progesterone (Camacho-Arroyo et al., 2011).

In males, serum progesterone concentration has been found to predict 65% of the variability in serotonergic 5HT_{1A} receptor binding (Lanzenberger et al., 2011), the study awaits replication. Low expression of 5HT_{1A} receptors has been found in depression and anxiety disorders (Akimova et al., 2009).

Whilst synthetic progestins exist in most contraceptive and hormone replacement medications there is to date only one published study in female rats of the effects on the brain. Four week treatment with ethinylestradiol, levonorgestrel or both showed that levonorgestrel reduced cortical levels of progesterone and allopregnanolone by over 60% and was associated with reduced exploratory behaviour (Porcu et al., 2012). Little or no research has been done in humans (Brinton et al., 2008).

1.3.4.3 The Progesterone Receptor

The progesterone receptor is one in the family of so called steroid receptors of which the androgen receptor, estrogen receptors, orphan receptors and vitamin D receptors are other examples. The progesterone receptor (PR) has 2 major isoforms A and B of which A is a truncated form of B. A third form C is found in humans and is thought to regulate transcription of A and B. The receptor is coded by a single gene at chromosome 11q22-23 (Mani, 2008). Progesterone receptors are located intracellularly and are bound to so called chaperone proteins such as heat shock protein. When progesterone comes into contact with the PR- chaperone protein complexes the chaperone protein dissociates and the now ligand bound progesterone receptor interacts with specific response elements in target genes (Brinton et al., 2008). This is the classical genomic action of progesterone receptors. However second messenger system activation by neurotransmitters, growth factors and neurosteroids (progesterone and others) can also activate the PR system. Different second messenger systems can activate the dissimilar PR dimers of AA or BB or AB and the response with respect to which genes are transcribed will thus differ (Mani, 2008).

Progesterone receptors are expressed in many brain regions, not just sexually dimorphic areas, and in some areas they appear to be more numerous in males than females, possibly accounting for men's greater sensitivity to progesterone (Wagner, 2008). Those responsive to oestrogen regulation are in sexually dimorphic regions whereas those who do not respond to oestrogen are in cerebellum and cortex (Mani, 2008). Progesterone receptors have in the human adult been detected in entorhinal cortex and subiculum with autoradiographic techniques (Sarrieu et al., 1986) yet a post-mortem study failed to find intra-nuclear PR (or intra nuclear Estrogen Receptors for that matter) within cortex (Bezdicikova et al., 2007). Yet given the findings that PR is found in dorsal raphe, hypothalamus and pituitary at least in monkeys (Bethea, 1994; Kohama et al., 1992) and there is widespread distribution in rats and guinea pigs in hippocampus, frontal cortex, centeromedial amygdala, thalamus, caudate, cerebellum and spinal cord (Brinton et al., 2008) it seems likely the same is true for humans. It has been suggested that progesterone receptors during pregnancy appear to be regulated by *maternal thyroid hormones* rather than by oestrogen and other sex hormones (Wagner, 2008). The high progesterone environment during foetal life appears necessary for motor development and maturation of the foetus as evidenced by benefits of progesterone supplementation after extreme preterm delivery of the infant (Trotter et al., 2001).

Pharmacologically, a potent progesterone receptor antagonist is mifepristone or RU486 which has an effect on progesterone receptors at doses much lower than those required for glucocorticoid receptor blockade (0.5-1mg/kg compared with 4-6mg/kg) (Spitz and Bardin, 1993). Mifepristone has in phase 2 and 3 studies been given in high doses yielding antiglucocorticoid effect to persons with psychotic depression without major effect on depressive symptoms (Nihalani and Schwartz, 2007), perhaps unsurprising as another study examining high dose treatment with both this and a newly developed specific progesterone receptor antagonist revealed *increased* immobility in rats suggesting depressive type behaviour (Beckley et al., 2011) once again reminding us of the possibility of a bell-shaped dose response curve.

As to gene variants in the progesterone receptor, one polymorphism has been associated with increased risk for panic disorder in women (Ho et al., 2004).

1.3.4.4 Progesterone – Psychiatric Disorders and Behaviour

Schizophrenia:

Two small studies have reported higher progesterone in males with schizophrenia compared to healthy controls (Taherianfard and Shariaty, 2004) when subjected to metabolic stress (Breier and Buchanan, 1992) but age was not taken into account. Another study also found higher serum progesterone (Guest et al., 2011) but unfortunately the sexes were analysed together and it was unclear whether women were taking oral contraceptives.

Mood Disorders:

Progesterone has long been suspected of having depressogenic effects in humans given the occurrence of premenstrual dysphoria in some women at a time of very high progesterone concentrations. A number of studies have examined links and possible mechanisms and the interested reader is referred to a comprehensive review in this area by Andréen (2009). In summary, evidence has been found for changes in GABA_A receptor sensitivity to allopregnanolone and to the rapid change in progesterone concentrations in the premenstrual period in women with premenstrual dysphoria.

A single injection of progesterone in women during follicular phase which brings progesterone and allopregnanolone concentrations in serum to luteal phase concentrations has been found to increase amygdala response to fearful faces (van Wingen et al., 2008) whilst coupling of the amygdala with the medial prefrontal cortex also was increased. It was hypothesized that this coupling may correlate with a tendency to ruminate on negative events. Yet when progesterone was increased further there appeared to be *reduced* neural response to fear in the amygdala and a shift to hippocampal activity in memory encoding (van Wingen et al., 2007). Another study failed to find luteal phase differences in women with premenstrual dysphoria but did find evidence for greater amygdala reactivity as a function of follicular phase concentrations and as a function of anxiety proneness (Gingnell et al., 2012). Even in healthy men there appear to be correlations with fear and anxiety whereby state but not trait anxiety scores correlate with progesterone concentrations (Brambilla et al., 2004).

With regard to bipolar disorder, luteal phase progesterone (7-10 days prior to menses) was noted to be higher in euthymic bipolar women than controls (Hardoy et al., 2006) however given the uncertainty of ascertaining just these days in the cycle and the large variability of progesterone peaks it is hard to draw conclusions from this result. Progesterone during follicular phase or in males has not been investigated in bipolar disorder.

Low progesterone during follicular phase or postmenopausally in women with chronic fatigue syndrome was found to be inversely proportional to depression scores and it was found that a metabolite, isopregnanolone, was significantly correlated with the occurrence of chronic fatigue, suggesting alteration in progesterone metabolism (Murphy et al., 2004).

1.3.4.5 Effects of Allopregnanolone in the Brain

Allopregnanolone is the endogenous antagonist of DHEAS with inhibitory activity at the $\sigma 1$ receptor (Wang et al., 2007) as well as agonistic allosteric effects at the GABA_A receptor (Agis-Balboa et al., 2007; Miczek et al., 2003) (see Figure 3). This means that there is prolongation of chloride channel opening time with greater influx of chloride into the cell when the receptor is activated; yet the effect is bell-shaped such that both low doses and very high doses may have less effect than intermediary doses. Behaviourally this effect can also

be seen with other GABA_A agonists such as benzodiazepines or alcohol where low or high doses may result in aggression or proconvulsant effects in place of the more intermediary sedative effects. The picture is further complicated by the effects of the 3 β metabolites of ALLO which counteract the effect of ALLO but when this is not present, take over as GABA_A positive modulators (Stromberg et al., 2006). Changes in allopregnanolone concentration also change the expression of receptor subunit types within the GABA_A receptor which in turn give contradictory effects (Follesa et al., 2004) further complicating the interpretation of in vivo effects.

Acute administration of allopregnanolone lessens firing of serotonergic neurons whereas more chronic administration increase firing rates (Robichaud and Debonnel, 2004). With regard to dopamine signalling allopregnanolone has been shown to have no effect in cortical regions in adulthood but if given early in the postnatal period in rats ALLO did produce changes in the striatum (Muneoka et al., 2009). Behavioural effects consistent with reduced anxiety are seen if ALLO is infused into medial prefrontal cortex or amygdala (Engin and Treit, 2007), components of the fear response pathways.

Like progesterone, allopregnanolone has neuroprotective effects by means of reducing NMDA mediated excitotoxicity in human neuronal cell lines (Lockhart et al., 2002), increasing neural progenitor cells in rats and human cell lines (Wang et al., 2005) and reducing build up of β -amyloid plaques in addition to reducing microglia activation in rat models of Alzheimer's disease (Chen et al., 2011). However if allopregnanolone was given late in the first postnatal week to rats (equivalent to late foetal stage in humans) then a reduction of overall cell numbers, but not of glutamatergic cells, was seen later in the adult thalamus suggesting a role also in cell pruning (Gizerian et al., 2004). These findings are similar to those seen in schizophrenia possibly indicating a contribution of neurosteroid systems to this disorder.

1.3.4.6 Allopregnanolone – Psychiatric Disorders and Behaviour

Schizophrenia:

ALLO has been measured in a very small number of males with schizophrenia where a linear relationship was found between allopregnanolone and overt hostility during the previous week as well as to paranoia subscale scores on PANSS (Spalletta et al., 2005).

Mood Disorders:

As seen in Figure 4 an inverse U shape or bell shaped curve exists for negative mood symptoms and serum ALLO concentrations in postmenopausal women treated with progestins (Andreen et al., 2006). CSF Allopregnanolone and progesterone concentrations were seen to be lower in males with major depression yet only the former normalized with fluoxetine treatment (Uzunova et al., 1998). A blunted allopregnanolone response to stress has been seen in women who had a history of depression (Klatzkin et al., 2006) while the converse has been noted in panic disorder (Strohle et al., 2003). Additionally both progesterone and allopregnanolone have been noted to be elevated in males with panic disorder compared with healthy controls (Brambilla et al., 2005) possibly a compensatory attempt at endogenous anxiolysis as women however with another anxiety related disorder, post traumatic stress disorder have shown lower CSF allopregnanolone concentrations (Rasmusson et al., 2006). It is important to note that whilst allopregnanolone in the luteal phase is coupled to luteinising hormone it is much more intimately coupled to cortisol in other menstrual phases and in men (Genazzani et al., 2002) and is therefore, along with

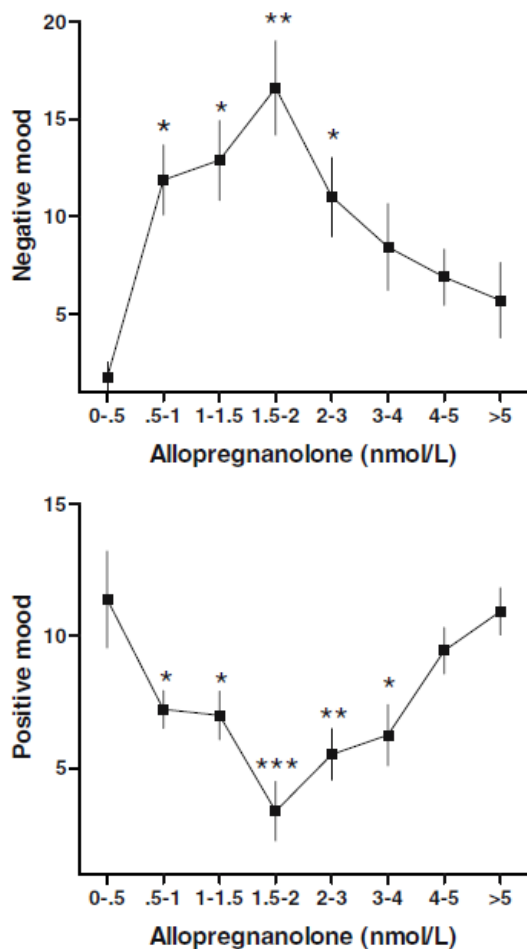


Figure 4: Mood symptoms according to serum allopregnanolone concentrations in postmenopausal women receiving hormone replacement therapy (Andreen 2006)

cortisol and progesterone, stress responsive. There are as yet no published studies examining allopregnanolone in bipolar disorder. In summary, progesterone and ALLO are involved in endogenous anxiolytic effect and in mood states in a biphasic, bell shaped manner. They act agonistically via GABA_A receptors in much the same manner as benzodiazepines and alcohol. They are thought to be the endogenous antagonists of DHEAS and DHEA. Progesterone also acts at the PR with genomic effects and second messenger cascades, some of which are implicated in schizophrenia and in the treatment of bipolar disorder. Whilst progesterone concentrations vary widely in women across the menstrual cycle and during pregnancy, male brains are more sensitive to progesterone and associations are found with differences in the serotonergic system. Thus, the previously held assumption that it is not relevant to study progesterone concentrations in males due to the low levels compared to females can be regarded as erroneous.

1.3.5 Testosterone

1.3.5.1 Factors Affecting Testosterone Concentrations

In blood, there is often an age related decline in free testosterone (Thilers et al., 2006) though clinical reference ranges do not take age into account and it is argued that we require the same testosterone concentration throughout life (Brambilla et al., 2007). Testosterone is strongly bound to sex hormone binding globulin (SHBG) the result of which is to make the bound testosterone unavailable to cells. The percentage bound to SHBG is circa 60-70% in women and 40-50% in males. Some of the remaining testosterone is loosely bound to albumin (approx 50% for men, and 30% for women) making it available to the body. The remaining testosterone is regarded as freely circulating (free testosterone). The testosterone portion that is free and that which is bound to albumin are together described as bioavailable testosterone and is regarded as that available for cellular uptake. Peak testosterone concentrations in the blood are found between 7 and 9 am (Diver, 2006).

Because SHBG sequesters testosterone, factors influencing its concentration are paramount in the control of testosterone availability. SHBG is manufactured in the liver, testes, placenta and brain (within neurons) (Damassa and Cates, 1995). Thyroxine increases SHBG by reducing hepatic lipogenesis and the stimulation of hepatocyte nuclear factor 4 (HNF4)

(Selva and Hammond, 2009). In contrast, prolactin, insulin and insulin like growth factor all reduce SHBG synthesis as does sugar (Selva et al., 2007). As a result low SHBG concentration (and by corollary high free testosterone or bioavailable testosterone) can be seen as a marker for diabetes and metabolic syndrome in women at least (Selva et al., 2007). In males low SHBG accompanied by *low* testosterone have been associated with insulin resistance and metabolic syndrome (Grossmann, 2011). Oestrogen increases SHBG whilst low concentrations of testosterone reduce it. Surprisingly, higher concentrations of testosterone do not appear to induce production of SHBG (Edmunds et al., 1990), generating a positive feedback system in cellular availability of testosterone rather than a homeostatic system at higher concentrations. SHBG concentrations in women are viewed to be relatively heritable (0.56) (Coviello et al., 2011). SHBG is increased in the luteal phase of the menstrual cycle whereas total testosterone remains unaltered by menstrual phase meaning that both free testosterone and bioavailable testosterone fall during the luteal phase (Schijf et al., 1993) although the extent of this is not large. *SHBG* gene is found at 17p12 and differing numbers of copy number repeats have been associated with lower risk of having diabetes type 2 (Perry et al., 2010), and polymorphisms have been linked to structural changes in the protein leading to reduced binding to testosterone (Ohlsson et al., 2011) and to lower concentrations of SHBG (Cousin et al., 2004) making more testosterone available to cells as for example in PCOS (Damassa and Cates, 1995).

Previously it has been thought that testosterone is reduced by medications such as neuroleptics that raise prolactin. Hyperprolactinemia is associated with lower luteinising hormone concentrations which in turn are associated with lower serum testosterone in men and with lower estrogen in women (Wilson, 1998). In males, some have found *increases* in testosterone in the first 30 days of treatment with neuroleptics (Brambilla et al., 1975) yet others have found no association between neuroleptic treatment with moderate doses haloperidol or risperidone (Kaneda and Fujii, 2000; Kaneda and Ohmori, 2003) or sulpiride (Baptista et al., 1997) and testosterone. Only in very high dose treatment in males has testosterone been noted to decrease (Brown et al., 1981; Rinieris et al., 1989). Rat experiments have confirmed that high dose but not intermediate dose treatment with neuroleptics reduces testosterone in males (Okonmah et al., 1986). The effect in women is less well known - healthy women given moderate doses of sulpiride for 30 days showed no effect on free testosterone concentrations yet oestradiol was reduced (Baptista et al., 1997). No studies have yet documented before and during neuroleptic treatment hormone concentrations in women with schizophrenia or bipolar disorder.

The neuroleptics mentioned above have all prolactin raising properties yet neuroleptics without this profile have also been associated with altered testosterone concentrations (Huber et al., 2005). Olanzapine treatment has for example been found to reduce SHBG concentrations in women but not in men (Birkenaes et al., 2009). A number of studies have shown that there are additional control mechanisms which mean an uncoupling between dopamine, prolactin and androgen synthesis (see for example Connell (1984), Molitch (1993)). Prolactin lowers SHBG synthesis generating an increase in bioavailable testosterone (Misra et al., 2004) but to complicate the picture further is the finding that when dopamine is lowered by, for example, neuroleptics *testosterone* begins to *stimulate* prolactin secreting cells (Christian et al., 2000). Presumably this explains the initial increases seen in testosterone but that after a while other mechanisms operate to effect the changes seen.

Several other medications have been suggested to affect serum concentrations. Sodium valproate has been shown to increase testosterone early on in treatment both by reducing

SHBG and increasing testosterone in males (Rattya et al., 2001b) and in women (McIntyre et al., 2003; Rattya et al., 2001a). Yet these studies have examined values against healthy population means (not healthy controls) or those of lithium treated persons and have not followed levels before and after treatment initiation. Lithium has itself been suggested to *reduce* testosterone in male rats (not female) (Allagui et al., 2006; Sheikha et al., 1987) but not in humans (Hunter et al., 1989). Antidepressants such as amitriptyline and imipramine have been associated with reduced serum testosterone concentrations (Przegalinski et al., 1987) but citalopram and mianserin increased them. The brain concentrations did not follow suit. The mood stabilizer lamotrigine has not in humans been shown to change concentrations yet carbamazepine has been suggested to increase androgen receptor (AR) signalling as shown in hippocampal tissue after surgical resection in complex partial seizures (Stephen et al., 2007). With regards to other psychiatric treatments ECT does not appear to have any acute effect on testosterone (Cooper et al., 1989).

Maternal smoking has been found to change testosterone concentrations in foetal rat brain due to inhibition of aromatase (Sarasin et al., 2003). In fact it was found that there were sex specific modifications in testosterone concentration which appeared in some situations to persist into adulthood. Male rats became, rather surprisingly, demasculinized in behaviour (Segarra and Strand, 1989) but without changes in plasma testosterone concentrations. Female rats however, had raised testosterone concentrations persisting into adulthood (Smith et al., 2003). If given to males in adulthood nicotine appeared to reduce testicular steroidogenesis (Jana et al., 2010; Kavitharaj and Vijayammal, 1999). In women, cigarette smoking has been associated with increased free testosterone (Brand et al., 2011).

Alcohol reduces testosterone rapidly and transiently in males (Ida et al., 1992) yet the converse is true for women. In fact red wine, as opposed to white wine, increases testosterone and reduces SHBG in women (Shufelt et al., 2011). Alcohol dependent women have in several studies been shown to have raised free testosterone due to reduced SHBG (Pettersson et al., 1990; Valimaki et al., 1990; Valimaki et al., 1995) while men have had normal testosterone (Schiavi et al., 1995) until complications set in. Hence both alcohol and smoking are associated with contrary effects on testosterone in males and females.

1.3.5.2 Effects in the Brain

Testosterone has a number of actions in the brain most of which are mediated by the binding of the androgen receptor whose actions will be explored in greater detail below. This discussion focuses on the effects of testosterone at physiological concentrations, not on the effects of anabolic steroid use. In the research paradigm which focuses on gonadectomy and testosterone substitution one attempts to distinguish the androgenic effects of testosterone from the effects of its metabolite oestradiol by giving finasteride (a SRD5A2 antagonist) which blocks conversion of testosterone to the more effective androgen dihydrotestosterone (DHT) thereby increasing instead the conversion to oestradiol. Predictably, there are dosing effects of testosterone – at physiological concentrations there are clear neuroprotective effects which are reversed into apoptotic effects when supraphysiological doses are given as in the case of anabolic steroid abuse (Estrada et al., 2006; Nguyen et al., 2005b). Testosterone has been found in many parts of the brain in both sexes and perhaps surprisingly without sex difference (Lanthier and Patwardhan, 1986). Foetal testosterone is responsible for the rightward asymmetry in the development of the corpus callosum (Chura et al., 2010). The highest adult concentrations are found in the substantia nigra, preoptic area and hypothalamus however testosterone is also found in significant concentrations in the amygdala, caudate, putamen, thalamus and in the hippocampus as well as in temporal and

parietal cortex (Bixo et al., 1995). In human females, hippocampus and temporal cortex are more testosterone rich than estrogen rich (Bixo et al., 1995). In areas where testosterone and androgen receptors are not found, testosterone can still exert effects, for example, testosterone's stimulation of vasopressin (Magnusson and Meyerson, 1996) is mediated through central amygdala pathways relaying onto bed nucleus stria terminalis (BNST) and subsequently to the paraventricular nucleus of the hypothalamus (Viau et al., 2001).

Testosterone increases neurite outgrowth (Estrada et al., 2003) via increased neuritin gene expression (Marron et al., 2005), increases spine density in CA1 layer of male rat hippocampus and maintains synapses in this same region (Hajszan et al., 2008; Leranthe et al., 2003; Leranthe et al., 2004). Combined with brain derived neurotrophic factor (BDNF) it maintains dendritic arborisation (Yang et al., 2004). It is essential for example, in astrocytic differentiation and sprouting in the hypothalamus (Mong and McCarthy, 1999), and has been found to increase GFAP immunoreactivity in astrocytes in the hippocampus (Conejo et al., 2005). GFAP is thought to be essential in maintaining the shape of astrocytes. In a similar vein testosterone is also involved in maintaining pyramidal neuron soma (body) size (Isgor and Sengelaub, 1998).

Vasodilatory effects of testosterone have been noted in the periphery as well as in the CNS (Azad et al., 2003; Rosano, 2000; Zitzmann et al., 2001) and low testosterone has in a number of studies been found to be a significant risk factor for cardiovascular ill health in males (Yeap 2010) as well as for diabetes and for the forerunner of both – metabolic syndrome (Zitzmann, 2009).

1.3.5.3 The Androgen Receptor

The androgen receptor (AR), like the progesterone receptor, is one in the family of steroid receptors. It was initially deemed to be a nuclear receptor. It is coded for by the gene *AR* located at Xq12. A membranous form has since been found (Foradori et al., 2008). The nuclear receptor is responsible for the genomic effects of testosterone - the bound AR acts as a transcription factor binding to specific DNA response elements causing activation or repression of transcription and protein synthesis (Foradori et al., 2008).

AR Gene:

There are 8 exons in *AR* of which the first exon corresponds to the N terminal domain in the protein, numbers 2 and 3 to the DNA binding domain and the remainder to the ligand binding domain. Over 400 non lethal point mutations have thus far been identified in the *AR* gene and associated with reduced receptor activity. Unsurprisingly, these have predominantly been identified in males investigated for infertility and poorly developed genitalia and secondary sex characteristics. Many of these are known as partial or complete androgen insensitivity syndromes. Associations with prostate cancer (commonly) and with liver cancer (some) have also been noted in males (<http://androgendb.mcgill.ca/>). Their effects in females have not been examined although premature ovarian failure has been mentioned (Panda et al., 2010). Some mutations yield an androgen receptor which binds less strongly to testosterone or DHT and more strongly with DHEA, progesterone and oestradiol thus enabling non androgens to exert androgenic effects in the body (Tan et al., 1997).

The genetic variations that have been most studied are those in exon 1: copy number variants of CAG repeats and GGN repeats. The first repeat sequence of CAG has normally a length between 8 and 35 repeats (Rajender et al., 2007). Short repeat sequences have been

associated with higher serum testosterone concentrations in women (Brum et al., 2005; Westberg et al., 2001) and men (Krithivas et al., 1999; Mifsud et al., 2001), hirsutism and acne in women and androgenic alopecia in men (Sawaya and Shalita, 1998). Yet other studies have found higher free and total testosterone in men with greater numbers of repeats (Crabbe et al., 2007; Lindstrom et al., 2010; Manuck et al., 2010; Walsh et al., 2005) and some studies have found no relation at all (Canale et al., 2005; Schatzl et al., 2003; Van Pottelbergh et al., 2001). The discrepancies may be due firstly to the practice in all but one study to split the number of repeats into short and long based on a mean or median split and, secondly, that different racial groups have different number of repeats such that 21-23 alleles may be classed as long in one study and short in another. Recent data indeed suggest optimal activity at around 22 repeats and 20% less activity at 16 or 28 repeats (Nenonen et al., 2010). With regards to clinical associations, repeat sequence variability has been associated with a number of traits and some psychiatric conditions for example, adolescent depression (Geng et al., 2007; Su et al., 2007), adolescent aggressiveness (Vermeersch et al., 2010) and muscular tension (Jönsson et al., 2001).

Between the above repeat sequences is a synonymous base substitution polymorphism (rs6152) which has in a number of studies been found to predict hyperandrogenism in men and in women. Interestingly, hyperandrogenism is coupled to the G allele in males (see meta-analysis by Zhuo et al., 2011) but the A allele in females (el-Samahy et al., 2009; Peng et al., 2010).

Brain Androgen Receptor:

The brain AR exists as intra-nuclear and membranous forms. The cell membrane form appears to be most abundant in areas where the nuclear form is not prominent (DonCarlos et al., 2003). Membranous AR is found i) in dendritic spines of hippocampal pyramidal cell and granule cells, as well as ii) in synaptic vesicles within preterminal axons and axon terminals of the GABAergic stratum in the cortex (Finley and Kritzer, 1999), and iii) in gap junctions with astrocytes (Tabori et al., 2005). It is almost 100% co-localized with tyrosine hydroxylase expressing cells in the nervous system, at least in rats (Kritzer, 1997). It activates tyrosine kinase (Kousteni et al., 2001) enhancing downstream the MAPK pathway causing cell migration, proliferation and differentiation. Androgens have been found to interact with the glutamatergic system to boost dopamine release in the prefrontal cortex (Aubele and Kritzer, 2011). Additionally, testosterone reduces dopamine turnover in the nucleus accumbens (Yang and Shieh, 2007) and changes D1 receptor binding in the striatum (Andersen et al., 2002). Testosterone upregulates the binding potential of the dopamine transporter (Kindlundh et al., 2002) as well as increasing dopamine and serotonin concentrations in the above areas in the brain (de Souza Silva et al., 2009). Androgen receptor activation upregulates monoamine oxidase type A gene expression (Ou et al., 2006) contributing to effects in the monoaminergic systems in the brain.

The nuclear form is often co-localized with somatostatin containing cells in amygdala, cortex, hippocampus, hypothalamus and BNST. It does not co-localize with corticotrophin releasing hormone, vasopressin or oxytocin containing cells, exerting its effects on these via interneurons (Viau et al., 2001).

1.3.5.4 D2:D4 Digit Ratio and Prenatal Testosterone Exposure

Grüning in 1886 measured finger length from metacarpal joint to tip of fingers and found a sex difference whereby males had a lower ratio of index finger (D2) to ring finger (D4) length compared with females (Peters et al., 2002). Whilst several other investigators

demonstrated similar findings the results were largely forgotten until Manning (1998) reawakened interest by describing possible relationships between these ratios and sex hormones especially testosterone. It was proposed that prenatal testosterone exposure affected the expression of the Hox gene family shown to influence growth and patterning of digits in animals and humans. Since then over one hundred studies have examined D2:D4 digit ratios as a proxy for prenatal testosterone exposure. Whilst studies agree that there are sex differences, racial differences are larger (Manning et al., 2003). It has been found that adult testosterone concentrations are not related to D2:D4 ratios (Hönekopp et al., 2007; Muller et al., 2011). In line with development hypotheses, it is thought that not only prenatal but also perinatal and neonatal testosterone concentrations may all influence the D2:D4 finger ratio, at least in males (Knickmeyer et al., 2011). Confirmation of a correlation between foetal amniotic fluid testosterone and D2:D4 ratio has been forthcoming in one study (Lutchmaya et al., 2004) yet another study found little correlation between amniotic testosterone concentration and foetal blood concentration of testosterone (Rodeck et al., 1985). Maternal cord blood concentrations of androgens have not been found to correlate with D2:D4 ratio (Hickey et al., 2010). Even with the above caveats there have been studies done, for example in congenital adrenal hyperplasia, showing masculinised patterns of digit ratios in affected women compared with healthy controls (Brown et al., 2002; Okten et al., 2002; Puts et al., 2008) adding circumstantially to the hypothesis that there is an association between digit ratios and pre/perinatal testosterone.

With regard to psychiatric disorders, male schizophrenic patients have shown feminized patterns of D2:D4 ratios. In fact while there was no difference in ratios between male and female patients, female patients had the same digit ratios as healthy females (Arato et al., 2004; Collinson et al., 2010). Similar findings have been seen in adolescents meeting criteria for schizotypal disorder (Walder et al., 2006) regarded by many as part of the schizophrenia spectrum of disorders. Several studies have examined autistic children and found increased masculinised pattern (Manning et al., 2001; Milne et al., 2006; Noipayak, 2009) however in all of the autism studies age ranges were substantial and results difficult to interpret as digit ratios alter over time in childhood and may not correlate with adult values. (Bloom et al., 2010; Knickmeyer et al., 2011). In fact the only study thus far examining digit ratios in adults found a slightly more feminized pattern in males and no difference in women (Bejerot et al., 2012). There have been no published studies to date examining digit ratios in bipolar disorder or other affective disorders.

Measuring digit ratios have been done by several methods making comparison between studies difficult. Grüning (1886) and Pfitzner (1893) measured from the flexed metacarpal joint the outstretched fingers on the dorsal aspect. Manning (1998) measured the ventral length of the fingers as measured from the most proximal crease of the finger to the finger tip using callipers. This measurement can be obtained either live or via photocopies (Peters et al., 2002) and is the method most commonly used in modern studies.

1.3.5.5 Associations with Behaviour and Psychiatric Disorders

Given the observation that anabolic steroids can increase aggressive behaviour (review (Kanayama et al., 2010)) and produce manic like symptoms (Gruber and Pope, 2000; Pope and Katz, 1988) a number of groups have investigated whether endogenous concentrations of testosterone are correlated with these and other behavioural or psychological symptoms.

Fear and Anxiety:

Functional imaging studies examining response to exposure to angry and fearful faces have found that low testosterone concentrations in hypogonadal males and, to a certain extent, in females are associated with relatively little activation in orbitofrontal cortex (OFC) and anterior cingulate (Redoute et al., 2005; Stanton et al., 2009) but greater amygdala activation, an effect thought to be due to GABA_A disinhibition. Other studies have documented greater amygdala activity in those with high normal testosterone (Derntl et al., 2009; Hermans et al., 2008; Manuck et al., 2010; van Wingen et al., 2009) suggesting that both extremes of testosterone concentrations activate fear pathways. Anxiogenic situations normally result in increased delta - beta coupling of EEG in the OFC and cingulate (Knyazev, 2011; Knyazev et al., 2006) an effect which is blunted by high testosterone (Schutter and van Honk, 2004). Furthermore, short sequences of CAG-repeats in *AR* have been associated with the greatest amygdala activation (Manuck et al., 2010). These subtle differences in brain activation may interact with the brain effects of cortisol to affect behaviour. For example, utilising computer games, it has been found that men who had high concentrations of both testosterone and cortisol chose not to compete again after defeat whereas those who had high testosterone but low normal cortisol competed again. Those with low testosterone were less likely to compete again regardless of cortisol concentration. Defeat reduced testosterone in the high testosterone males but had little effect in those with low concentrations (Mehta and Josephs, 2010). This has been interpreted to mean that testosterone is involved in responsiveness to social threats and social hierarchy, a finding that may have significance for the equivocal findings of the role of physiological levels of testosterone on aggression (Archer, 1991; Brooks and Reddon, 1996; Dabbs et al., 1987; Popma et al., 2007; Rada et al., 1976; Rada et al., 1983; Rasanen et al., 1999). The possible interactive effects of testosterone, social environment and cortisol may be important also for the interpretation of low testosterone concentrations found in several psychiatric disorders.

Severe stress associated with military training has been found to reduce testosterone (Opstad, 1992) even though exercise on its own increases concentrations especially in older males (Ari et al., 2004). Lower CSF testosterone (free testosterone) has been found in males with post traumatic stress disorder even though serum levels were normal. Normally there is no direct relationship between corticotrophin releasing hormone and testosterone however in this group of PTSD sufferers there was an inverse relationship between the two (Mulchahey et al., 2001).

Depressive Symptomatology:

A number of studies have documented the association between low serum testosterone in healthy males with depressive ideation (see review by Amiaz and Seidman, (2008) and Vermeersch (2010)) yet it is not nearly so certain that one can extrapolate this to say that major depression can result from low testosterone. Blunted morning testosterone has been observed in depressed males in some studies (McIntyre et al., 2006; Rupprecht et al., 1988; Schweiger et al., 1999; Steiger et al., 1991) but not in all (Levitt and Joffe, 1988; Rubin et al., 1989). CSF testosterone was *higher* in unmedicated males with dysthymia and in those with an unspecified depression not meeting criteria for major depression compared with other males with substance use or major depression who had made a suicide attempt (Gustavsson et al., 2003). In women, the only study examining the potential association between depression and testosterone found that testosterone was *elevated* in major depression and normalized with treatment (Baischer et al., 1995). Other studies examining women suffering from hirsutism have found a greater occurrence of elevated testosterone in depressed women compared with those who were not depressed (Shulman et al., 1992) and correlations have

been seen between depressive symptoms, hostility and phobic anxiety and free testosterone (Derogatis et al., 1993). However, arguing against these findings is an open labelled trial in depressed women with testosterone gel, which raised testosterone levels to high normal, and which found that women with the *lowest* pretreatment testosterone concentrations had the greatest antidepressant effect of the testosterone gel (Miller et al., 2009) with one third of these previously treatment resistant patients achieving remission at 8 weeks and a further third obtaining >50% symptom reduction on MADRS. In animal studies, a sex difference has been seen with regards to effectiveness of testosterone treatment on depressive type symptomatology. Male gonadectomised rats responded to testosterone as well as to imipramine and showed an augmentation effect of testosterone on hippocampal cell proliferation. Female gonadectomised rats responded to neither of these interventions but aromatase inhibitors were not administered which makes it difficult to determine whether effects were the result of testosterone or estrogen or an imbalance of the two (Carrier and Kabbaj, 2012).

Schizophrenia:

Hypogonadism has been documented in a large subgroup of male and female patients with schizophrenia before the era of neuroleptics, also being associated with a range of structural abnormalities in the adrenal glands (Hoskins and Pincus, 1949; Lewis, 1921; McCartney, 1929). Males with schizophrenia have in a number of small modern studies been shown to have lower serum testosterone concentrations (total and or free or bioavailable) (Brambilla et al., 1975; Fernandez-Egea et al., 2011; Huber et al., 2005; Mendrek et al., 2011; Rasanen et al., 1999) and a correlation has been found with the severity of negative symptoms (Akhondzadeh et al., 2006; Goyal et al., 2004; Ko et al., 2007; Shirayama et al., 2002) and to disorganization but not to negative symptoms or depressive symptoms (Fernandez-Egea et al., 2011). Treatment with exogenous low dose testosterone gel in a randomized active / placebo trial has shown effect on negative symptoms in males (Ko et al., 2008). Even in a subgroup of high risk adolescent males with prodromal symptoms suggestive of schizophrenia development, salivary testosterone (which measures free testosterone) has been found to be low when matched for age and Tanner stage (van Rijn et al.). Males with schizophrenia who had attempted suicide had by far the lowest testosterone concentrations compared with other male schizophrenic patients (Tripodanakis et al., 2007). A possible exception to the above is the suggestion that testosterone is more normal in males with the paranoid subtype schizophrenia (Mason et al., 1988). Few studies have examined women with schizophrenia however some have suggested higher free and bioavailable testosterone concentrations (Ataya et al., 1988; Johansson and Mowry, 1996; Mendrek et al., 2011) without a change with neuroleptics (Oades and Schepker, 1994).

Bipolar Disorder:

The only study comparing males with bipolar disorder to males with schizophrenia found that manic males had higher testosterone serum concentrations however there were no healthy controls to compare with (Ozcan and Banoglu, 2003). No studies yet published have compared testosterone in females with bipolar disorder to healthy controls, rather the studies have focussed on comparing women on and off sodium valproate, given the assertion that valproate increases the risk for polycystic ovarian syndrome (McIntyre et al., 2003; Rasgon et al., 2005a; Rasgon et al., 2005b). It is in fact not clear if there solely are links between valproate and PCOS or if the increased risk for PCOS is also linked to the underlying bipolar disorder as seems to be the case with epilepsy (Akdeniz et al., 2003).

Cognition and Personality:

The link between testosterone and cognitive performance in healthy adults has been investigated with regard to understanding sex differences in verbal and spatial performance and yielded ambiguous results. For example, low testosterone in women has been associated with better verbal fluency in younger women (Thilers et al., 2006) but the reverse in older women (Drake et al., 2000). In males and females higher testosterone has been linked to better spatial memory (Aleman et al., 2004; Cherrier, 2005; Postma et al., 2000), better mental rotation ability in females (Postma et al., 2000) and to faster reaction times (Fontani et al., 2004) yet another study found that low testosterone in males were associated with better performance on block design, a test of visuospatial ability (Yonker et al., 2006). In adolescent females higher free and bioavailable testosterone has been linked to aggressiveness (Pajer et al., 2006). Total testosterone in women has in one study been found to correlate with paranoia traits on the Minnesota Multiphasic personality inventory (Avgoustinaki et al., 2012).

Developmental Findings:

Of relevance in understanding the role of early hormonal milieu in brain development predisposing to later psychiatric morbidity are the animal experiments which either stress pregnant rats or subject very young animals to stress. Restraint stress in pregnant dams has the effect of reducing the adult testosterone concentration in their male offspring although early postnatal testosterone concentrations are normal. Monoamine levels in adulthood are altered in sexually dimorphic ways dependent it seems upon the effect of testosterone and oestrogen in the brain. Female offspring's brains were more masculinised than those whose mothers had not been stressed. In females, prefrontal cortex dopamine was reduced and its metabolite homovanillic acid (HVA) increased as was norepinephrine and the serotonin metabolite 5HIAA. However in the hippocampus both dopamine and its metabolite HVA were reduced. In males only dopamine was reduced in the prefrontal cortex and hippocampus (Bowman et al., 2004). Another study found that high neonatal exposure to testosterone in female rats increased the likelihood of in adulthood exhibiting depressive and anxious behaviour when exposed to chronic mild unpredictable stress (Seney et al., 2012).

That stress effects on hormonal parameters can transmit into second generation offspring has now been documented in a study which only stressed pat grandmothers of male rats. These male second generation offspring were demasculinized in their brain gene expression patterns and showed amongst other findings increased ER β receptors (Morgan and Bale, 2011). Female offspring appeared not affected.

In summary, testosterone has a number of effects in the brain including genomic actions as well as roles in regulating dopaminergic transmission and other monoaminergic actions as shown for example by being co-expressed with tyrosine hydroxylase in neurons and interacting with GABAergic and glutamatergic systems. There is clinical evidence of it being reduced in males with chronic schizophrenia and in males with depressive symptoms. Several studies point to the possibility of it being raised in females with the same conditions. Whilst chronic neuroleptic treatment does not appear to raise concentrations of testosterone in males the question has not been well examined in women. Intriguingly, there appear to be long term effects of stress on adult testosterone if pregnant animals are stressed, going in opposite directions in male and female offspring whereby males become feminized and females masculinised. Testosterone affects behaviour as well as frontal-amygdala activation pathways in response to stress, with differential effects according to cortisol concentrations. D2:D4 ratio as a proxy for pre and perinatal testosterone has not been examined in relation to

AR polymorphisms and repeat sequences. There is conflicting data as to what foetal measure of testosterone D2:D4 ratios relates to even though foetal androgenising syndromes have been associated with differences in these ratios.

1.3.6 Summary of Neurosteroid Effects

From the above it can be seen that neuroactive steroids have a wide array of functions in everything from neurodevelopment, maintenance of cell and regional architecture, neuroprotection as well as maintaining balance in the glutamatergic and the GABAergic systems thus providing a basis from which to influence monoaminergic and dopaminergic systems in the brain. The major effects of relevance to psychiatry are summarised in Table 6 on the next page. Some of these effects are sexually dimorphic in regions which are dimorphic whereas others cut across sex and are similar in males and females. Whilst the knowledge of the hormonal effects in animals has grown rapidly in the last 20 years it has been decidedly less research done to document the effects in humans and to correlate structure with function by for example studying determinants of hormonal parameters in health and illness. The studies that have been done are often small in size and many lack controls and are of course beset with the usual difficulties of extracting illness effects from medication effects. The best documented findings hitherto appear to be low DHEAS and testosterone in males with schizophrenia along with the disturbances in the progesterone/allopregnanolone systems with premenstrual dysphoria. The effect of prenatal stress on altering the expression of important steroidogenic enzymes in brain in animals would lead one to think that similar changes may be evident with prenatal stress in humans whether or not this is in the form of emotional stress, cigarette smoking or other conditions engendering oxidative stress and that this would be an important area for further research.

Table 6: Summary of Effects of Neurosteroids				
	DHEA(S)	Progesterone	Allopregnanolone	Testosterone
Factors affecting metabolism	Genetic factors account for large portion of variability, ↓ mirtazapine, clozapine and lithium ^a	A large range of medications – ↑ fluoxetine ^b , mirtazapine, desipramine, carbamazepine, olanzapine ^a	A large range of medications – ↑ fluoxetine ^b , mirtazapine, desipramine, carbamazepine, olanzapine ^a	Factors affecting SHBG concentrations – thyroxine, obesity, insulin concentrations; smoking and alcohol – males ↓ females ↑, valproic acid ↑
Neurogenesis and Neuroprotection	Acts via stimulation of growth factors	Reduced inflammation, maintenance myelin	Increases neural progenitors, reduces NMDA mediated neurotoxicity	↑ spine density and astrocytic differentiation, maintains cell body size and architecture of astrocytes
Oxidative stress	Reduces oxidative stress	Possibly antagonistic of estrogens protective role	Unknown	Reduces oxidative stress
NMDA receptor	stimulation via sigma receptor: agonists- fluoxetine, citalopram, venlafaxine, imipramine, cocaine, metamphetarmine	None	inhibition via sigma receptor – other antagonists haloperidol, trifluoroperazine	None
GABA _A receptor	Inhibition	None	stimulation	None
Other mechanism of action		Progesterone receptor ^c as well as intracellular cascades (MAPK, Akt, protein kinase C, calmodulin dependent protein kinase)		Androgen receptor - intranuclear – genomic effects
Dopamine system	enhances D1 functioning and presynaptic release	Unknown	Unknown	↑ dopamine release, changes to COMT and MAOA activity
Monoamine system	↑ adrenal catecholamine synthesis and release, ↑ postsynaptic noradrenaline release	unknown	↓ firing serotonergic neurons acute but habituation effects seen	changes to COMT and MAOA activity
Psychiatric associations	DHEA ↑ with acute stress, DHEAS ↓ schizophrenia males, unknown women	Change in GABA _A receptor sensitivity to prog. Allo in PMDD ^d , stress responsive	Bellshaped curve negative mood symptoms acc to dose, increased in women with panic disorder, stress responsive	Low and high normal concentrations – ↑ amygdala activation, ↓ OFC activation, ↓ in men with schizophrenia, depressive symptoms, unknown women

^a effects documented in rats, ^b dependent upon social rearing environment, ^c antagonist RU486 (Mifepristone) – at doses lower than glucocorticoid receptor blockade,

^d Premenstrual dysphoric disorder

2 AIMS OF STUDIES

In light of the expanding literature showing central roles in organization, neuroprotection and activation of neurotransmitter systems it was hypothesized that several of these neurosteroids may be implicated in the pathophysiology of bipolar disorders. More specifically it was reasoned that as DHEAS and progesterone/ALLO exert opposite effects on the GABA and glutamate systems in the brain and have been implicated in conditions that involve anxiety, dysphoria as well as mania that these may be also altered in bipolar disorder especially in those who exhibit mixed episode characteristics. Additionally the evidence suggesting a role of testosterone in psychosis raised the possibility that this may also be altered in persons with psychotic subtype of bipolar disorder.

Specifically the aims of the studies were

Study 1: To compare euthymic bipolar patients who had exhibited previous manic/hypomanic irritability to those who had not in relation to i) serum concentration of progesterone, and ii) to common (SNPs) in the three genes *HSD3B2*, *SRD5A1* and *AKR1C4*; and to study these SNPs in relation to serum progesterone concentrations.

Study 2: To compare euthymic bipolar patients who had exhibited paranoid ideation with those who had not with respect to i) serum concentrations of DHEAS and progesterone, and ii) the allele frequency of common SNPs in the three genes *HSD3B2*, *SRD5A1* and *AKR1C4*.

Study 3: To compare euthymic bipolar patients with a history of depressive psychomotor agitation to those without with respect to i) serum concentrations of progesterone and testosterone, as well as ii) to polymorphisms in the three genes *AKR1C4*, *HSD3B2* and *SRD5A1*

Study 4: To determine whether euthymic female bipolar patients with a history of psychotic symptoms differed from bipolar women without psychosis and healthy controls with respect to i) rs6152 polymorphism of the androgen receptor gene, ii) in D2:D4 ratio, or iii) in current bioavailable serum testosterone, accounting for the effects of medication.

3 METHODS

3.1 Overview of Subject Selection and Assessments

The St. Göran Bipolar Project is a prospective, longitudinal study that was launched in 2005 at the Bipolar Affective Disorders Outpatient Unit of North Stockholm Psychiatric Clinic. The project aims to provide early identification, assessment, treatment and follow up of patients with bipolar disorder living in the geographical catchment areas of inner city Stockholm, Lidingö and some northwestern suburbs of Stockholm City. It serviced at the commencement of the study a population of 320 000 adults coming from affluent inner city areas, working class suburbs and impoverished areas with high numbers of immigrants and welfare recipients. Subsequently the North Stockholm Psychiatric Clinic has been split with the more impoverished areas now belonging to a separately owned and run outpatient clinic.

Patient Recruitment:

The research program was built into the clinical outpatient treatment program as a naturalistic study design in such a way that all patients were given the same basic workup regardless of whether they then consented to participating in the study (see flowchart of study design in Figure 5).

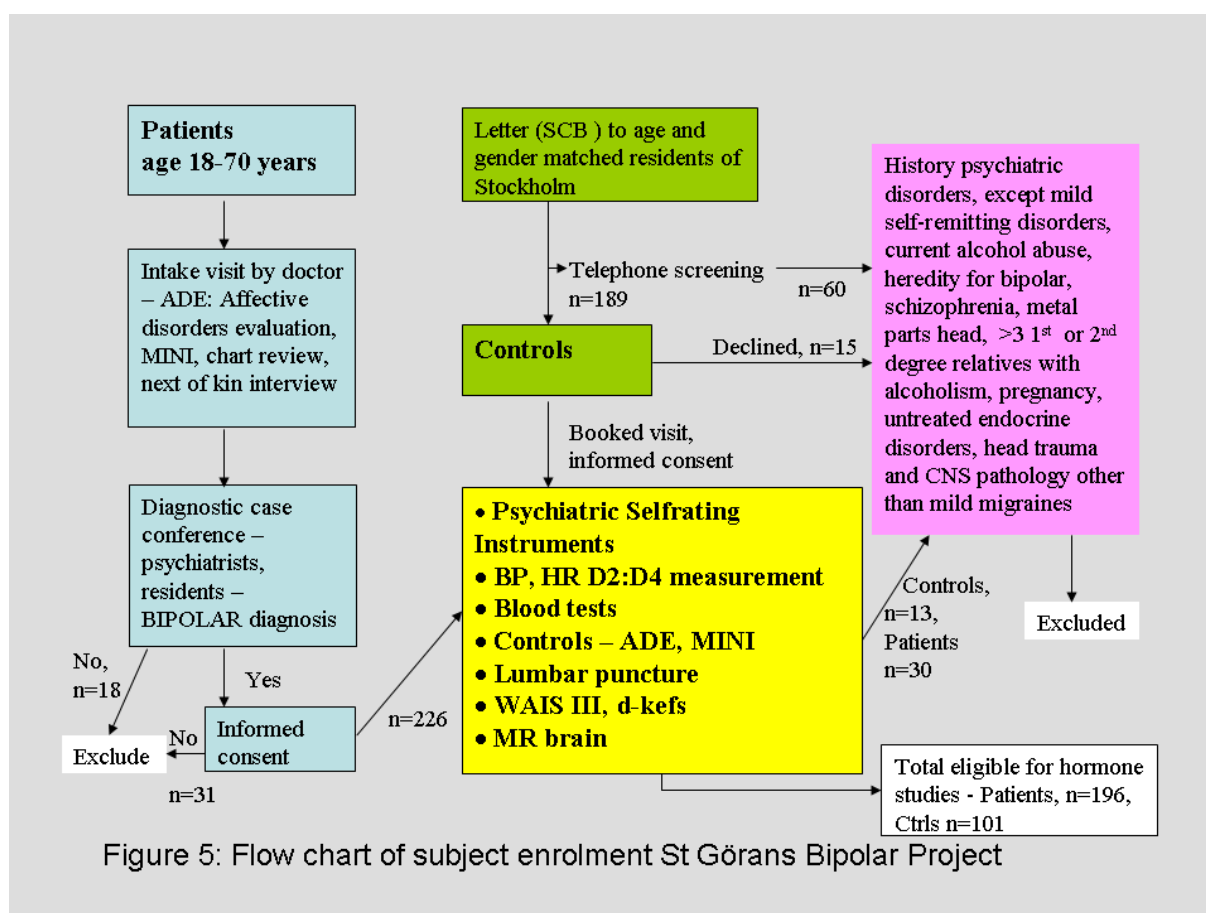


Figure 5: Flow chart of subject enrolment St Görans Bipolar Project

This clinical workup included in depth diagnostic interview by a psychiatrist or psychiatrist in training utilising the Swedish version of the Affective Disorders Evaluation (Sachs et al., 2003) which also required the taking into account of information gathered by chart review and history from relatives, use of the Mini-International Neuropsychiatric Interview MINI for ascertaining co-morbidity (Sheehan et al., 1998), as well as baseline blood tests and

measurement of blood pressure, height and weight. Data were presented at diagnostic conferences and diagnoses according to DSM-IV and according to Akiskal's classification were made by consensus of board certified psychiatrists and psychiatrists in training. If deemed to have a form of bipolar disorder then patients were approached to give consent to study participation. After consenting to the study, extra elements not critical to, but advantageous to, day to day management of their condition were added to their work up. These included a range of self-rating symptom schedules such as Alcohol use disorders identification test (AUDIT) (Saunders et al., 1993), Drug use disorders identification test (DUDIT) (Berman et al., 2005), structured clinical interview for DSM IV-tr axis ii personality disorders (SCID-II) personality screening (American Psychiatric Association, 1997), Swedish scales of personality (SSP) (Gustavsson et al., 2000) as well as lumbar puncture, structural MRI, as well as finger length of the 2nd and 4th digits in both hands. A subset underwent indepth interviewing for comorbid ADHD (see Ryden 2009) as well as complete neuropsychological assessment with Wechsler adult intelligence scale (WAIS-III) (Wechsler, 1999), the Delis-Kaplan Executive Function System (d-kefs) (Delis, 2001), as well as memory testing with Claeson-Dahl (Claeson, 1971). Clinically relevant information was fed back to the clinicians in order to tailor further investigation and treatment. The decision *to not participate* in the study did not preclude the work up of the patient with the above added tests; rather these components were dependent upon the individual clinician's determination of need according to the clinical picture for each patient. Depression symptom ratings with MADRS (Montgomery and Asberg, 1979) and mania symptom ratings using YMRS (Young et al., 1978) were conducted routinely for all patients at the clinic and were specifically ascertained by nurse and psychologist interview at the time of blood tests, lumbar puncture, MRI scan and neuropsychological evaluation for study patients. These examinations were not as a routine performed on the same day for the patients.

Healthy Control Recruitment:

Statistics Sweden (SCB) was asked to invite seven randomly selected persons living in the greater Stockholm area, age and sex matched, for each enrolled patient who had completed all parts of the workup, to participate in the study. Interested persons who did not themselves suffer from schizophrenia or bipolar disorder were asked to contact the study team and underwent a telephone interview by the study nurse in order to exclude other recurrent psychiatric problems, drug and alcohol abuse and heredity for bipolar disorder or schizophrenia. Those meeting inclusion criteria were booked in for a one day comprehensive assessment. The age matching was not done according to year of birth given the time lapse between recruitment of the 2 groups rather controls were matched according to the age at which the patient had undergone MRI examination. Response rate was 14%, matching other studies of similar nature according to Statistics Sweden. 59 were excluded at telephone interview mainly due to current illicit drug use (n=16), somatic ill-health (n=12), heredity in first degree relative of bipolar disorder or schizophrenia (n=9), metal precluding MR (n=9), ongoing mental health diagnoses (n=6), pregnancy (n=5), or moved out of area (n=2). Furthermore, 1 subject failed to show up for the day and 14 changed their mind about participating between the time of the telephone interview and the booked date. One person had no reason documented for their exclusion. Control subjects underwent a psychiatric interview for lifetime psychiatric pathology by experienced clinicians using MINI, which included the affective disorders section, as well as the same investigations the patients had undertaken including self-rating scales, psychological testing with WAIS-III, d-kefs and Claesson-Dahl, somatic tests, blood tests at 08-09 am, morning lumbar puncture and MRI of the brain. Because the psychiatric interview was expected to reveal some past pathology, case conferences were held between examining clinicians, primary investigator and study

coordinator to decide whether or not to include such persons in the study. It was thus decided to allow past minor depressive disorders, isolated mild episodes of panic disorder, eating disorders or obsessive compulsive disorder which remitted spontaneously or with short term psychotherapy. Substance abuse was screened for at telephone interview by the nurse, in the psychiatric interview, by AUDIT and DUDIT as well as by CDT in blood test. Overconsumption of alcohol as revealed by CDT or responses indicating large consumption (> 8 standard drinks per time more than 2 times per week), and/or amnesia and/or loss of control more than once per month resulted in the exclusion of these individuals from the studies presented here. Other exclusion criteria were neurological conditions other than mild migraines, untreated endocrinological disorders, pregnancy, dementia, recurrent depressive disorder, and suspected severe personality disorders (based on interview, SCID2 and SSP screening) that additionally had heredity for schizophrenia, bipolar disorder or substance use in more than 3 first or second degree relatives. Remuneration was given to control persons.

3.2 Ethics of Study

Consent to the study was given by patients during a period of euthymia both verbally and in writing after obtaining written information about the study. Opportunity was given to reflect over the implications of the study. Participation in the study did not alter in any way the suggested treatment options or the availability of particular treatments. Baseline investigations were all carried out during euthymic periods. Importantly treatment was not withheld during investigation periods. Patients were informed that withdrawal from the study could occur at any time without disadvantaging them in way of treatment or care provided. However they were also informed that data obtained prior to withdrawal could be still used in studies. Controls had had an overview of study information sent to them with the letter from SCB. They were provided with opportunity to ask questions at the telephone interview as well as before signing the informed consent on the day of the study. The study was approved by the Stockholm Regional Ethics Board 2005 and with an addendum 2009 for the inclusion of healthy controls. The study was conducted in accordance with the latest protocol of the Helsinki Declaration on research ethics.

3.3 Specific Methodology Studies 1-4

In all studies inclusion criteria for the study was bipolar 1 or 2 diagnosis. Participant numbers vary between studies 1 to 3 in line with the ongoing further inclusion of research subjects to the project and due to missing hormonal data, principally early in the history of the project. In studies 1-3 the design was case-case, in study 4 healthy controls were added to a case-case design to make 3 groups.

Symptom Considerations:

The ADE uses a binary system of symptom classification of most symptoms commonly associated with bipolar disorder and detailed symptom definitions are provided in each of the papers that make up this dissertation. A rating of present utilizes all sources of information that are available. A copy of the English version of the ADE is available at <http://www.manicdepressive.org/images/blankade.pdf>. Psychomotor agitation as opposed to retardation was chosen as a variable to be studied for paper 3 as it is most commonly recalled by patients and usually well documented in case notes making the rating possibly more reliable than that for retardation. Psychotic or non psychotic ratings for paper 4 were based on a history of hallucinations or delusions. Information on thought disorder and disorganized behaviour is not recorded in the ADE and could therefore not be determined. This lack would underestimate the number of persons who had in fact been psychotic. It was decided to include one female in the psychotic group who had had symptoms only during a drug

induced psychosis at age 15 and now in her twenties had not yet had psychotic symptoms during mood episodes. It was argued that because not everyone who takes drugs experiences psychotic episodes, there is likely to be an underlying biological vulnerability to psychosis in those that do. It was not possible from the data available to grade the severity or duration of the psychotic experiences. Patients were deemed to be euthymic at blood test if they scored <14 on nurse administered MADRS and < 14 on YMRS.

SSP Ratings of Aggression, Irritability, Somatic and Psychic Anxiety:

The SSP is a revised version of the Karolinska scales of personality tested in population as well as clinical samples. It has 91 items divided into 13 subscales. There are 4 response alternatives ranging from disagreeing completely to agreeing completely. Each subscale consists of 7 items rated 1-4 which were added to give subscale scores. As the study was case-case design and no control subjects were included in studies 1-3 scores were left untransformed.

D2:D4 Ratio:

Second digit to fourth digit length ratio was calculated for each hand utilising Grüning's method: holding fingers at 90° flexion at metacarpal joints measuring posteriorly from end of bone to tip of second and fourth digit thereafter dividing the D2 length by the D4 length.

Hormone Sampling:

Baseline blood tests were sampled from stable euthymic outpatients and from healthy controls in 10 ml EDTA tubes between 0800-0900h. Chemoluminescent methods were used for all hormone analyses. For detailed methodology of analyses see the specific papers. Bioavailable testosterone was calculated using subjects own albumin, SHBG and total testosterone according to the validated formula provided by www.issam.ch/freetesuit.html (Vermeulen et al., 1999). Bioavailable testosterone as opposed to total testosterone or free testosterone was chosen as reflecting most closely the testosterone that is available for cellular uptake. All men and women regardless of menstrual phase or contraceptive status were included for DHEAS determination. All men but only premenopausal women in the follicular phase of the menstrual cycle and not receiving hormonal contraceptives were included in the progesterone analyses. In study 3 all men and all women not taking hormonal medications were included for the testosterone evaluation as was the case for women in study 4 for the analysis of testosterone.

Genetics:

There were no published studies examining polymorphisms in *AKR1C4*, *HSD3B2* and *SRD5A1* with respect to psychiatric or hormonal differences at the point in time when polymorphisms were to be selected. This meant that SNP's were selected from NCBI SNP database that had a European population frequency of 0.10 or above, had $r^2 \leq 0.8$ with each other and that were spread throughout the gene. They were not tagged with each other. The population frequency was later checked against the Wellcome Trust consortiums SNP frequency in European populations with full agreement. Rs6152 was selected as an AR polymorphism that has been documented to be associated with hyperandrogenism. Genetic analytic techniques are described in detail in the papers. As a quality control every 10th sample was replicated. All men and women regardless of age who had been included in the St Görans bipolar project by a certain date were included in the genetic analysis. This however meant that not all of the patients and controls eligible for inclusion in the hormonal studies presented here had genetic data available. The ethnic origin of all the patients was caucasian European as ascertained by asking the patient's ethnic origin 3 generations back.

Stratification according to parental genotype was not possible due to age span of probands which restricted the availability of parents.

Statistics:

Basic demographic data were analysed in Statistica Release 8 using student's t-test, χ^2 or Mann-Whitney statistic as appropriate to the type and distribution of the variable to be studied. Progesterone and DHEAS were normally distributed in men and in the women sampled in follicular phase. Bioavailable testosterone was normally distributed in males but exhibited a log normal distribution in women in paper 3. With the addition of control subjects the distribution of bioavailable testosterone now best approximated a gamma distribution. General linear models (ANCOVA using a combination of categorical and continuous variables) were used to analyse differences in serum progesterone, DHEAS and, in males, bioavailable testosterone with respect to symptom, or specified alleles with age as a covariate as pronounced age effects were seen in all the hormones. Confounders were ascertained and controlled for. In women bioavailable testosterone was analysed using a log normal generalized model including age and BMI as covariates in paper 3 and according to gamma distribution in paper 4. Odds ratios and significance testing using χ^2 were calculated for the specified alleles using the UNPHASED 3.1.4 program (Dudbridge, 2003). The Hardy-Weinberg equilibrium (HWE) was checked for each SNP using PLINK v1.07 (Purcell et al., 2007). To avoid type II error in these small studies with limited power the decision was made to adhere to Perneger's proposal (1998) and account for all tests of significance performed but do not correct for multiple testing. Perneger argues that when testing specific biological or syndromal systems the calculations performed are not random events but associated with each other whereby nesting of symptoms and findings occur. In applying corrections such as Bonferroni which assumes randomness significant associations are lost. A clinical example would be testing serum blood sugar and lipids concentrations and assuming that these would be independent of diabetes.

4 RESULTS

Table 7: Summary of studies 1-4

	Study 1	Study 2	Study 3	Study 4
Research question	Is there a difference in progesterone with prior irritable mania/hypomania? Is there a genetic predisposition?	Is there a difference in DHEAS or progesterone if previous persecutory ideation during mania/hypomania? Is there a genetic predisposition?	Is there a difference in testosterone or progesterone if one had had depressive psychomotor agitation in bipolar disorder? Is there a genetic predisposition?	In women, is there a difference in bioavailable testosterone if one has bipolar disorder or a psychotic form of it? Is there an effect of genetic predisposition or of foetal testosterone? Are there treatment effects?
Design	Cross-sectional Case-case	Cross-sectional Case-case	Cross-sectional Case-case	Cross-sectional Case-case and case-control, women
Results	Men with past irritability had lower progesterone and this was associated with the missense variation in <i>AKR1C4</i> ; no relationship with trait irritability or comorbid anxiety disorders	Men exhibited low progesterone and DHEAS. Risk alleles for irritability in men were protective of risk to develop paranoid ideation in women. One minor allele in <i>HSD3B2</i> increased risk of paranoia in men. No relationships with trait suspiciousness.	In men who had been irritable during mania, depressive agitation increased progesterone relative to irritable only. Both sexes – polymorphisms in <i>SRD5A1</i> increased risk for agitation. No relationships with trait anxiety.	A allele rs6152 <i>AR</i> doubled risk of psychosis. Masculinised D2:D4 ratio in early psychosis debut regardless of debut age of bipolar disorder. No contribution of psychosis on bioavailable testosterone when controlling for effect of neuroleptics, age and BMI. A but not G allele associated with lower SHBG only in presence of neuroleptics.
Conclusion	Differences in neurosteroid synthesis may predispose men to developing irritable type mania, there may be genetic vulnerability	Risk alleles for men appear protective in women. Low DHEAS and progesterone may predispose men to both irritability and to persecutory ideation in mania	Unlike the previous symptoms of mixed mania where sex differences were noted in neurosteroid genetics, both sexes exhibited vulnerability to depressive phase agitation	Indices of early androgenisation and hyperandrogenism associated with psychosis; current testosterone increased as a result of neuroleptics but only in those with polymorphism in <i>AR</i>

4.1 Study 1

The study examined serum progesterone and polymorphisms in the 3 genes coding for the regulatory enzymes in progesterone metabolism as a function of whether or not 71 males and 107 females with a bipolar 1 or 2 disorder had only had irritable mood in their manic or hypomanic episodes.

The main finding was that men who had exhibited irritable mood as opposed to euphoric mood had substantially lower serum progesterone (Figure 6).

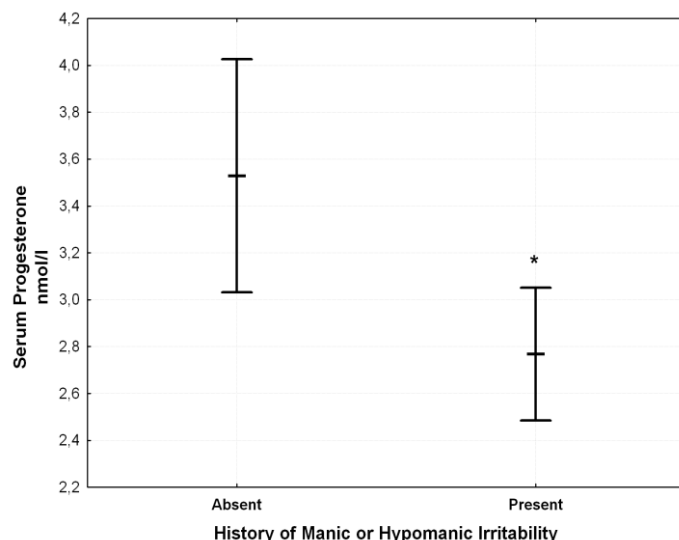


Figure 6: Euthymic serum progesterone concentration in males as a function of past irritability during mood elevation when age controlled for. $F=7.05$, $p=0.0099$. Vertical bars denote 95% confidence interval

Additionally, although group size was a limiting factor, there was a five-fold increased risk in males only of having been irritable if one had variant alleles in *AKR1C4* (Table 8). The small group size of non irritable males having the variant alleles means a high degree of uncertainty in the odds ratios as reflected by the borderline significant p-values. A significant difference in progesterone concentrations could also be noted in those males with the missense mutation in *AKR1C4* (Figure 7).

Significantly, whilst irritable mood during mood elevation was strongly associated with trait irritability on the SSP in males (19 versus 14, $p=0.004$) there was no significant association

Table 8: Odds Ratio of Having Experienced Manic or Hypomanic Irritability in Relation to Single Nucleotide Polymorphisms

Gene	SNP	Allele frequency ^d	Allele ^e	Females		Males	
				χ^2	Odds ratio (95% CI)	χ^2	Odds ratio (95% CI)
AKR1C4 ^a	rs17306779	0.27 / 0.25	A/G	0.18	0.85 (0.42-1.72)	6.52 *	5.06 (1.07-23.86)
	rs3829125	0.16 / 0.19	C/G	0.01	1.03 (0.47-2.28)	4.03 †	5.44 (0.66-44.8)
	rs10904440	0.13 / 0.09	A/G	0.68	0.65 (0.22-1.89)	4.55 #	5.87 (0.73-47.06)
	rs12762017	0.10 / 0.15	G/C	0.08	0.87 (0.37-2.02)	0.04	1.13 (0.4-3.18)
HSD3B2 ^b	rs4659174	0.14 / 0.08	G/A	0.93	0.54 (0.14-2.08)	1.26	0.44 (0.09-2.15)
	rs2854964	0.32 / 0.27	A/T	2.42	0.58 (0.29-1.18)	0.03	1.07 (0.48-2.36)
	rs3765948	0.14 / 0.08	A/G	1.06	0.52 (0.13-2.00)	0.88	0.49 (0.1-2.43)
SRD5A1 ^c	rs8192139	0.48 / 0.40	C/G	0.40	0.81 (0.43-1.52)	0.10	0.87 (0.41-1.86)
	rs181807	0.45 / 0.49	A/G	0.12	0.90 (0.46-1.74)	0.08	1.12 (0.54-2.3)
	rs3822430	0.44 / 0.36	T/C	0.55	0.79 (0.40-1.56)	0.22	0.81 (0.32-2.01)
	rs3736316	0.30 / 0.35	G/A	0.39	1.23 (0.62-2.44)	0.02	1.06 (0.42-2.64)

SNP Single nucleotide polymorphism ^a Aldoketoreductase, type 4 ^b β -hydroxysteroid dehydrogenase

^c steroid 5 α reductase ^d European population frequency (Hap map published on NCBI SNP database)

followed by allele frequency in sample ^e Major allele followed by minor allele

* $p = 0.01$

$p = 0.03$

† $p = 0.04$

‡ $p = 0.05$

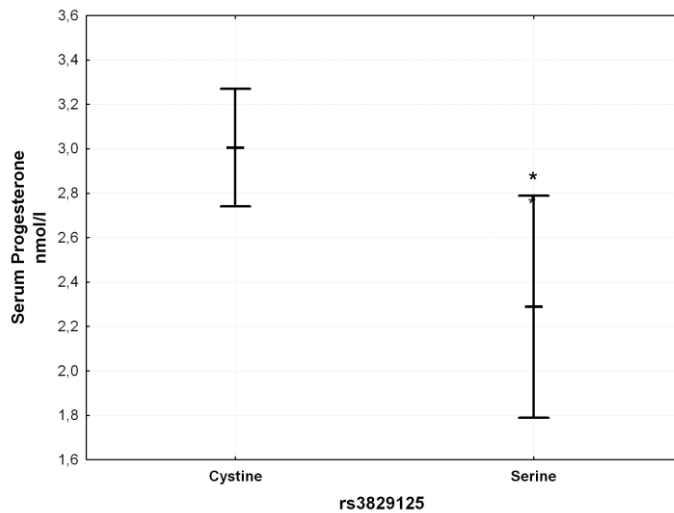
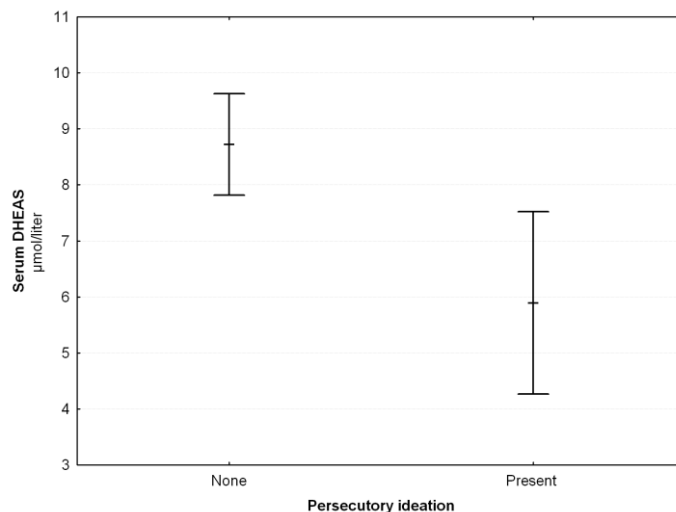


Figure 7: Serum progesterone concentrations controlled for age in euthymic males with bipolar 1 or 2 disorder according to amino acid at position 145 in AKR1C4 gene. *Covariate age 42.2 years.* Vertical bars denote 0.95 confidence intervals. * $p = 0.014$

between trait irritability and progesterone concentrations suggesting that the effect is coupled to some aspect of bipolarity rather than personality.

4.2 Study 2

The study examined serum progesterone and DHEAS along with polymorphisms in the 3 genes coding for the regulatory enzymes in progesterone metabolism and DHEAS synthesis as a function of whether or not 64 males and 96 females with a bipolar 1 or 2 disorder had exhibited persecutory ideation during mood elevation.



The main findings showed once again large gender differences. Firstly, hormone concentrations differed only in males where both DHEAS and progesterone were lower in those with a history of persecutory ideation (Figures 8&9). These findings had approximately 85% power.

Figure 8: Serum DHEAS concentrations in males as a function of past manic or hypomanic persecutory ideation adjusted for age (41.0years). Whole model adj $R^2 = 0.29$, $F = 7.30$, $p = 0.006$.

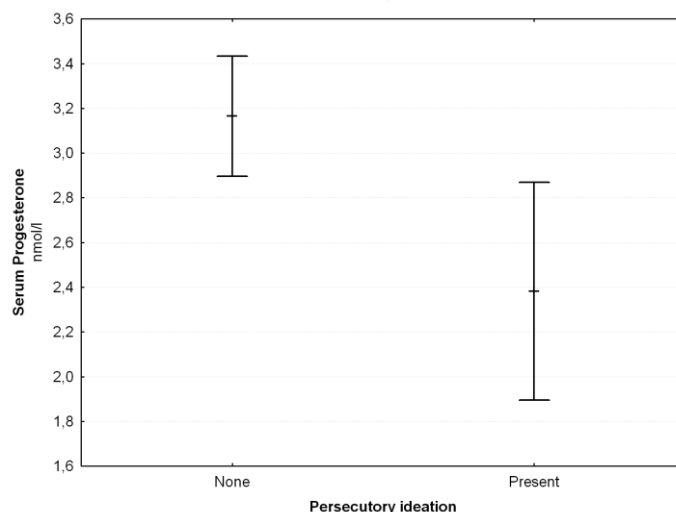


Figure 9: Serum progesterone in males as a function of past manic or hypomanic persecutory ideation adjusted for age (41.1 years). Whole model adj $R^2 = 0.31$, $F = 7.18$, $p = 0.009$.

Secondly the polymorphisms that in Study 1 had been associated with greater risk of irritable mania/hypomania in men were in women now *protective* of the risk of having shown persecutory ideation (Table 9). In fact the risk was reduced to 0.2, or in other words one fifth of the risk compared with the risk in those with the major alleles. The hormone differences in males appeared to come from sources other than the studied polymorphisms given the lack of associations found between the SNP's and the hormone concentrations. Yet there is a clustering of persecutory ideation with irritability which would partially account for the hormone findings (see section 4.5).

Table 9: Odds Ratio of Having Experienced Paranoid Ideation During Mania or Hypomania in Relation to Single Nucleotide Polymorphisms

Gene	SNP	Allele frequency ^d			Allele ^e	Females		Males	
		MM	Mm	mm		χ^2	Odds ratio (95% CI) ^f	χ^2	Odds ratio (95% CI) ^f
AKR1C4 ^a	rs17306779	93	55	9	A/G	3.59 *	0.51 (0.26-0.99)	0.55	1.4 (0.56-3.68)
	rs3829125	109	43	3	C/G	5.59 #	0.37 (0.15-0.94)	0.71	1.67 (0.52-5.35)
	rs10904440	123	28	2	A/G	0.22	0.78 (0.26-2.30)	0.01	0.93 (0.28-3.08)
	rs12762017	116	38	2	G/C	5.18 #	0.39 (0.18-0.87)	2.75 ‡	3.12 (0.68-14.3)
HSD3B2 ^b	rs4659174	128	25	1	G/A	2.77 ‡	2.70 (0.72-10.39)	3.97 †	0.31 (0.10-0.96)
	rs2854964	79	65	11	A/T	2.57 ‡	1.71 (0.86-3.38)	0.76	0.68 (0.29-1.60)
	rs3765948	130	24	1	A/G	2.66 ‡	2.69 (0.71-10.19)	2.69 ‡	0.36 (0.11-1.18)
SRD5A1 ^c	rs8192139	62	66	27	C/G	<0.01	0.99 (0.56-1.75)	0.02	0.94 (0.43-2.07)
	rs181807	39	81	37	A/G	0.07	1.08 (0.55-2.12)	0.14	1.16 (0.53-2.52)
	rs3822430	67	67	15	T/C	0.64	1.15 (0.62-2.12)	0.004	1.03 (0.42-2.50)
	rs3736316	66	64	18	G/A	0.08	0.91 (0.49-1.66)	0.12	1.16 (0.50-2.72)

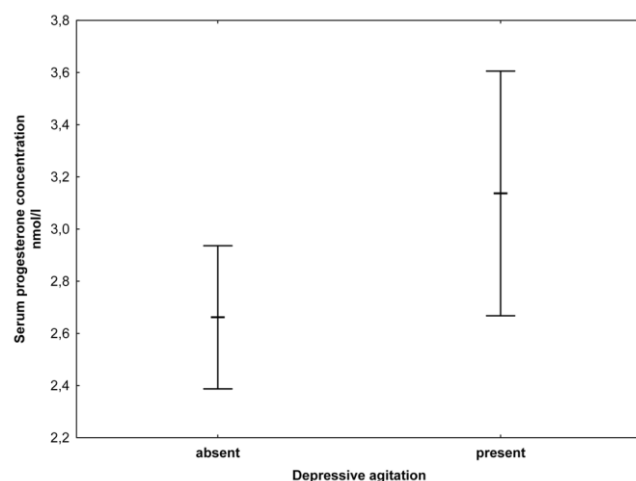
^a Aldoketoreductase, type 4, ^b 3 β -hydroxysteroid dehydrogenase, ^c steroid 5 α reductase, ^d Allele frequency in whole sample in absolute numbers, ^e Major allele followed by minor allele, highlighted alleles protective, ^f Odds ratio calculated for protective allele not for genotype

* p = 0.06 # p = 0.02 † p = 0.05 ‡ trend p = 0.09-0.10

4.3 Study 3

The study examined serum progesterone and bioavailable testosterone along with polymorphisms in the 3 genes coding for the regulatory enzymes in progesterone metabolism as a function of whether or not 69 males and 105 females with a bipolar 1 or 2 disorder had exhibited psychomotor agitation during depression.

The findings suggested that there was no sex divergence regarding the susceptibility to depressive psychomotor agitation and unlike the 2 previous studies, this was also reflected in that both sexes exhibited higher risk of agitation with gene variants in *SRD5A1* (Table 10).



There was a sex difference with regard to the effect of having been agitated on serum progesterone. Examining only the group who had shown manic irritability then those only showing irritability had lower progesterone than those with both symptoms (Figure 10).

Figure 10: Serum progesterone concentration in males with history of manic irritability as a function of past depressive psychomotor agitation Covariate - age 41.5 years. p=0.047.

Table 11: Odds Ratio of Having Experienced Depressive Psychomotor Agitation in Relation to Single Nucleotide Polymorphisms

Gene	SNP	Genotype ^d			Minor Allele Frequency ^e	Allele ^f	Males and females		
		MM	Mm	mm			χ^2	Odds ratio (95% CI)	
AKR1C4 ^a	rs17306779	105	56	9	0.23 / 0.25	A/G	0.62	0.76	(0.39-1.50)
	rs3829125	123	44	3	0.15 / 0.19	C/G	0.24	0.83	(0.39-1.74)
	rs10904440	134	31	2	0.10 / 0.09	A/G	0.39	0.75	(0.29-1.95)
	rs12762017	123	42	3	0.13 / 0.15	G/C	0.97	0.78	(0.17-3.60)
HSD3B2 ^b	rs4659174	139	27	1	0.09 / 0.08	G/A	0.01	1.05	(0.44-2.49)
	rs2854964	87	72	11	0.33 / 0.27	A/T	0.68	1.27	(0.71-2.30)
	rs3765948	142	26	1	0.11 / 0.08	A/G	<0.01	0.99	(0.41-2.35)
SRD5A1 ^c	rs8192139	60	54	21					
	non agitated								
	agitated	8	19	8	0.43 / 0.40	C/G	5.10 *	1.84	(1.07-3.16)
	rs181807	40	66	30					
	non agitated								
	agitated	4	21	10	0.47 / 0.49	A/G	3.28 #	1.63	(0.90-2.94)
	rs3822430	66	54	12					
	non agitated								
	agitated	9	18	6	0.38 / 0.36	T/C	5.95 **	2.00	(1.13-3.52)
	rs3736316	64	54	13					
	non agitated								
	agitated	9	18	6	0.30 / 0.38 [†]	G/A	5.50 *	1.94	(1.12-3.35)

^a aldoketoreductase type 1, 4th isoform gene, ^b 3 β -hydroxysteroid dehydrogenase gene ^c Steroid-5-alpha-reductase,

^d genotype in absolute patient numbers ^e European population minor allele frequency according to Wellcome Trust Case Control Consortium data followed by minor allele frequency in sample [†] minor allele frequency according to European HapMap database as SNP cannot be imputed from WTCCC, ^f Major allele followed by minor allele, risk allele is minor allele in all cases

**p=0.015 * p=0.02 #p=0.07

Because of insufficient numbers of individuals who had had persecutory ideation but been euphoric it was not possible to separately examine progesterone differences in that group using linear models.

Clustering of symptoms in patient sample St. Göran Bipolar Project (studies 1-3)

In view of earlier studies' findings of a separate irritability factor clustering sometimes with persecutory ideation, a breakdown of the symptoms considered in the first three papers was carried out. This showed strong associations in the patient group between both manic or hypomanic irritability and persecutory ideation during mania/hypomania as well as between manic/hypomanic irritability and depressive phase psychomotor agitation as well as overlap between all three symptoms (see Figure 11). The clustering of depressive agitation with manic phase irritability as opposed to depressive phase irritability has not been identified in factor analytical studies which have all focussed solely on either depression or mania not combined both sets of data. The low numbers of persons exhibiting all three symptoms unfortunately precluded the investigation of hormones and genetics in this particular group.

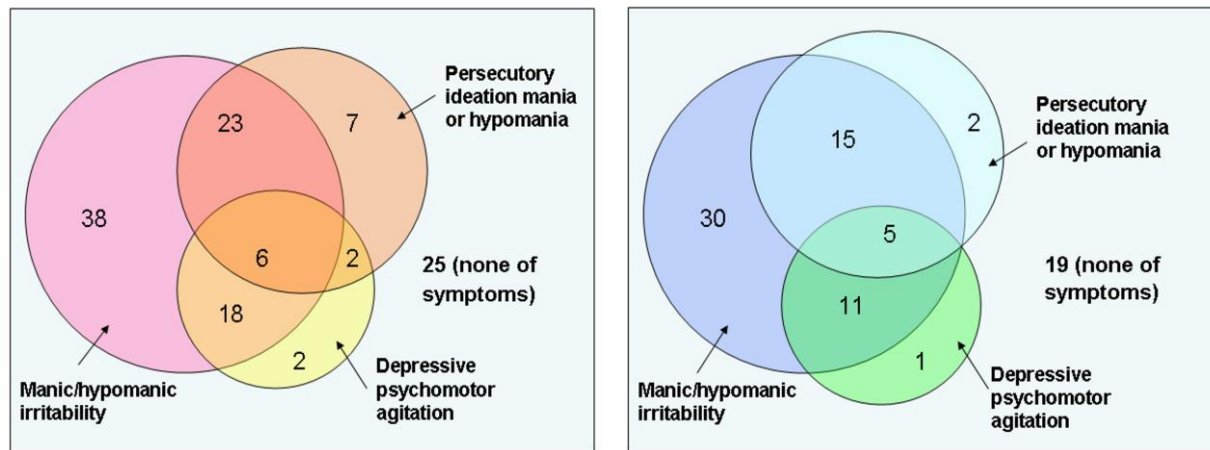


Figure 11: Symptom clustering in sample: Women in the left diagram, men in the right

4.4 Study 4

The study examined bioavailable testosterone along with a polymorphism in the androgen receptor gene known to be associated with hyperandrogenism in 68 women with a history of psychosis (defined as a history of hallucinations or delusions), 69 without such a history and 45 control women. Additionally a measure of foetal androgen exposure (D2:D4 finger length ratio) was analysed.

Due to there not being fully overlapping groups and the fact that only women who were not taking hormonal medications could be used for hormonal analysis, group sizes differ for the various parts of the study. The main findings were that the variant A allele rs6152 of the androgen receptor doubled the risk for having had psychotic symptoms of delusions and hallucinations (Table 12). An androgenised milieu as reflected in low D2:D4 ratio was associated with younger debut of psychotic symptoms (Figure 12) but not psychotic symptoms *per se*. Interestingly, the androgenising polymorphism was not associated with low D2:D4 ratio. A history of psychosis was not independently associated with bioavailable testosterone when adjusting for age, BMI and neuroleptics. Neuroleptics increased bioavailable testosterone, but only in those with the major G allele rs6152 (Figure 13). The explanation of this was not an increase in total testosterone but a reduction in SHBG concentrations (Figure 14).

Table 12: Allele Distribution rs6152 of Androgen Receptor Gene, Odds Ratio of Psychotic Symptoms if A Allele

	Allele distribution			A vs G allele	Odds ratio and
	AA	GA	GG	χ^2 , p value	95% Confidence interval
Psychotic bipolar	1	20	41	^a $\chi^2=5.36$, $p=0.02$,	2.56 (1.08-6.05)
Nonpsychotic bipolar	0	10	50		
Healthy controls	1	3	14	^b $\chi^2=4.67$, $p=0.03$	2.34 (1.07-5.12)

^a Psychotic versus non psychotic patients, ^b Psychotic versus all nonpsychotic

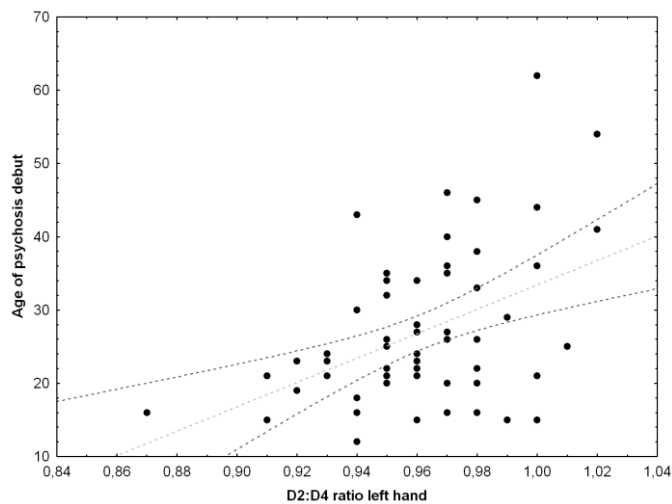


Figure 12: Age of psychosis onset in relation to left hand D2:D4 ratio. Male patterned ratio (<0.95) associated with early onset, $r=0.45$, $p=0.0003$

Figure 13: Interaction effects of rs6152 alleles with neuroleptic medication on bioavailable testosterone concentration in women. Overall Wald $X^2(1)=3.99$, $p=0.046$. **Dark grey bar to left non neuroleptic users, light grey bar to right neuroleptic users.** Spread of bars showing standard errors when adjusted for age and BMI. Difference between bioavailable testosterone concentration when only examining GG rs6152 - Wald statistic=15.97, $p=0.00006$.

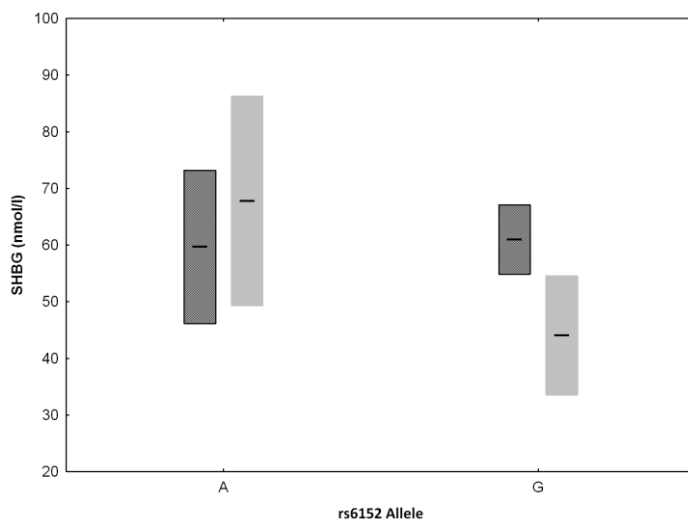
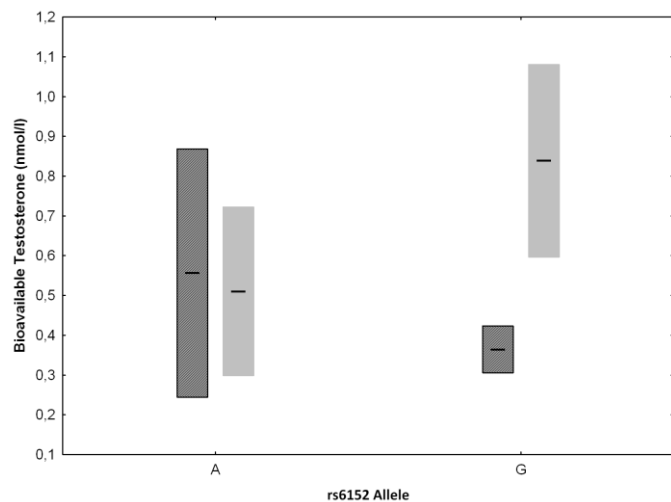


Figure 14: Interaction effects of rs6152 alleles with neuroleptic medication on SHBG Wald $X^2(1)=6.40$, $p=0.011$. **Dark grey bar to left non neuroleptic users, Light grey bar to right neuroleptic users.** Spread of bars showing standard errors when adjusted for age and BMI.

Supplementary graph (Figure 15) shows dosing effects of neuroleptics regardless of type on bioavailable testosterone (based on women not taking hormonal medications and not taking into consideration androgen receptor polymorphism).

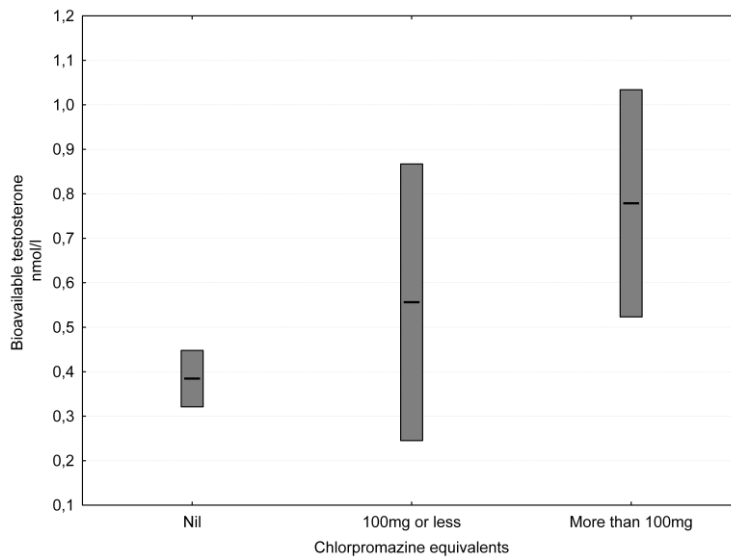


Figure 15: Effect of dose of neuroleptics regardless of type in all women controlling for age and BMI. $F=9.38$, $p=0.009$, $n=22$ with neuroleptics, $n=91$ no neuroleptics

Comorbidity:

The comorbidity of patients is described in Table 12. Seventy-six women had no lifetime history of anxiety disorders, 31 had one anxiety disorder and 26 had 2 or more anxiety disorders most commonly panic disorder with agoraphobia or social phobia. Fifty-four males had no anxiety disorder, 14 had one disorder, and 14 had 2 or more anxiety disorders. Forty-nine percent of men and 26% of women had a history of alcohol abuse or dependence. Interestingly, current self-reported abuse or dependence was reported by less than 10% in both sexes whereas current drug use especially of amphetamine and cocaine was more common in women than alcohol use. The cut-offs used for determining current abuse was 13-19 points in AUDIT for women and 15-19 in men. Likelihood of alcohol dependence was set at a score of >20 . For DUDIT scores >3 for women and >6 were used for men to determine abuse, dependence being set at >25 points. Significantly there was no association between progesterone and DHEAS with comorbid anxiety disorders in men.

Table 12: Lifetime Co-morbidity in Bipolar 1 and 2 Disorder

Diagnosis	Males (% in brackets)	Females (% in brackets)
Panic Disorder	18/82 (22.0)	50/138 (36.2)
Agoraphobia	6/82 (7.3)	13/138 (9.4)
Social Phobia	15/82 (18.3)	16/138 (11.6)
OCD ^a	9/82 (11.0)	17/138 (12.4)
GAD ^b	6/82 (7.3)	23/137 (16.7)
PTSD ^c	3/82 (3.6)	7/137 (5.1)
Anorexia Nervosa	0	16/137 (11.8)
Bulimia	1/82 (1.2)	20/136 (14.7)
PMDD ^d	0	44/137 (32)
MINI – alcohol abuse	13/57 (22.0)	21/105 (20)
MINI – alcohol dependence	15/57 (26.3)	7/106 (6.6)
MINI – drug abuse	12/56 (21.4)	11/102 (10.8)
AUDIT – alcohol abuse	3/57 (5.3)	6/106 (5.7)
AUDIT - alcohol dependence	1/57 (1.7)	3/106 (2.8)
DUDIT – drug abuse	3/54 (5.5)	13/105 (12.4)
DUDIT – drug dependence	1/54 (1.8)	2/105 (1.9)

^a Obsessive compulsive disorder ^b Generalized anxiety disorder ^c Post traumatic stress disorder ^d Premenstrual dysphoric disorder

5 DISCUSSION AND IMPLICATIONS FOR FUTURE RESEARCH

5.1 Studies 1-3

The first three studies examine symptoms associated with mixed affective states in bipolar disorder and their potential pathophysiological correlates with the neurosteroid system. Rather than attempting to find associations to the phenotypically diverse bipolar disorder syndrome focus is on teasing out potentially more significant phenotypes for further study by using a case-case method. In such a design one cannot make statements about what is common to all bipolar patients rather the approach enhances the possibility of finding differences between phenotypes. This is the necessary step before one can attempt to reverse phenotype (Schulze and McMahon, 2004). Reverse phenotyping is the process by which you use the discovered link between symptom and genes to then go back into the patient descriptions and examine each genotype (as opposed to diagnostic category) for symptoms frequency, clustering etc. One can thereby find new “symptom factors” or biomarkers associated with a purer phenotype which may very well cut across the diagnostic boundaries of systems such as the DSM. The need to do this is highlighted by the problems in finding consistent and universal pathophysiological correlates of the major diagnostic categories over the past 40 years.

As previously alluded to in the introduction mixed affective states have a strong background in the Kraepelinian axial system (Kraepelin, 1921) and have in modern times been recognized as constituting a more difficult to treat group which are made worse by antidepressants and respond differently to lithium and valproate (Koukopoulos et al., 2007). Yet epidemiological studies have shown that the symptoms seldom occur all at once as a syndrome rather it is most common with 1 symptom, second most common with 2, and so on (Maj et al., 2003). This prompted the DSM-5 committee to advise dropping mixed episode as an episode specifier and the classification will now include a course specifier of “mixed features”. In part these latter arguments agree with the symptom clustering pattern of the patients in these studies. Yet the interesting part is that disturbances in DHEAS and progesterone were found in men in all three symptoms examined - manic irritability and manic persecutory ideation both showing reductions and depressive agitation showing the reverse. This, combined with the albeit less clear-cut genetic data of increased risk of these symptoms when having variant polymorphisms in the genes coding for the steroidogenic enzymes, would suggest that there *is* a pathophysiological basis for regarding these as joined. Given that the gene frequencies were the same in our sample as a whole it appears unlikely that the genetic variants are involved in conferring risk to developing bipolar disorder per se, rather involved with the phenotype that is expressed.

Previous factor analytic studies of bipolar disorder in attempting to delineate phenotypes have failed to take into consideration the possibility that symptoms during one phase of illness may cluster more strongly with particular symptoms during the other phase than with other symptoms during the first mood state. The strong association of depressive psychomotor agitation with irritability during mania and not to depressive phase irritability in the bipolar patients here studied suggests the need for factor analytic studies that consider all symptoms during all phases of illness. Additionally there may be factors during euthymia such as persistent subsyndromal depression or cognitive deficits which may also cluster with particular symptoms. These are aspects that have not been taken into consideration in factor analytic studies or in the studies here reported. This approach may in fact yield a stronger

phenotype and may bear out the concept of Kraepelin's three axes or a third group of psychoses along the lines of Leonhard.

In these studies the differences that were found in hormone concentrations for men was surprisingly robust, average power being 80-90%. One has previously assumed that as women tolerate large swings in progesterone during the luteal phase males cannot be affected by variations from a low baseline, comparable to follicular phase concentrations in women. Yet research shows a much greater sensitivity in males to progesterone on for example EEG power (Fernandez-Guasti et al., 2003) making it likely that other control mechanisms operate in men than women. What these are have not yet been elucidated.

The finding that the same polymorphisms appeared to affect hormone levels differently in men and women and that at least AKR1C4 polymorphisms were risk alleles in men but protective of other symptoms in women highlight the necessity of analysing men and women separately when studying sex steroids and genetics. It means that extrapolation of results cannot be done between sexes. It also raises the possibility that some gene variants may be evolutionarily advantageous (for example by making women less suspicious) but that the price for this may be less advantageous in men.

The hormone findings in men with regards to persecutory ideation during mania could not be explained by the polymorphisms affected. Some of the variation may have been due to the AKR1C4 polymorphisms as irritability and paranoia overlapped yet there may be other mechanisms for the differences in concentration. Ideally one should have liked to have analysed the other metabolites in the pathways leading from progesterone to allopregnanolone and from pregnenolone to DHEAS. As serum is biobanked it is hoped that at least a subset of patients can be more fully characterized which can then shed light on which metabolites show build-up and which are lower. Utilizing this approach one can get some idea of the extent of enzyme activity. Combining this with gene expression studies (these enzymes are expressed in fat tissue as well as skin) and determination of gene polymorphisms one may get a much better idea of the functioning of the neurosteroid system.

The findings of differences in serum hormone concentrations during the euthymic phases of bipolar disorder show that there may be underlying differences in the steroidogenic system that may predispose to less capacity for internal soothing and reasoning when stressed. It has recently been shown that serum progesterone concentrations correlated positively to fMRI activation in OFC when processing pictures of both positive and negative facial images (Champagne, 2011) but only in men. Clinically it is common to find that patients with irritable subtype mania experience no subjective shift in mood, experiencing rather that people annoy them more and that they are being thwarted by others, experiences and feeling states that serve to further isolate them from others and to increase the irritability and the persecutory ideation. This becomes then a difficult positive feedback cycle. In order to break this cycle "soothing" influences need to come to bear on the system. If one recalls the work in rats where rearing in isolation generated differences in the neurosteroid system (specifically that of the DHEAS and allopregnanolone interactions with $\sigma 1$ receptor systems), as well as the differential role of antidepressants dependent upon rearing environment coupled to this system then one can begin to hypothesise about the interaction between early environmental influences and this particular phenotype. As in the recently reanalysed and expanded study where the effects of the serotonin transporter gene polymorphisms were magnified by childhood maltreatment in greatly increasing the risk for

recurrent depression (Uher et al., 2011), the neurosteroid system may exhibit similar properties which need to be addressed in future research.

The choice of studying hormone concentrations during euthymia does not address phase specific differences that are secondary to other mechanisms of which there are numerous possibilities given links between for example growth factors, oxidative stress and DHEAS and progesterone. However, it was thought that studying euthymic individuals would yield results of biological vulnerability having an etiological significance to the symptom under scrutiny. It would however be enlightening to see how the phase of illness affects these hormones and to correlate them with measures of oxidative stress or growth factors such as cytokines and BDNF which are phase responsive (Brietzke et al., 2009; Fernandes et al., 2011).

As all clinicians know, a person's personality colours the manifestation of the psychiatric disorder, and it was thus no surprise that the traits of irritability or mistrust correlated with the symptoms studied. However these traits were not the route by which steroidogenic differences were mediated. This is interesting as it seems to indicate the presence of separate pathways to particular symptoms which may reinforce each other.

In order to test whether the associations between sex steroids and symptoms are relevant to bipolar disorder per se or are associated with these symptoms in isolation it would be helpful to examine the same symptom complexes in other disorders (eg) persecutory ideation versus delusions of other types in schizophrenia, irritability and aggression proneness in schizophrenia, irritability and psychomotor agitation or retardation in unipolar depression. If associations are found then this may highlight a functional system that is separate from current nosological categories, much as the *G72* data has heralded a return to the neuroticism concept.

5.2 Study 4

The fourth study examined psychotic and nonpsychotic bipolar women as well as healthy control women. In this study it was found that there was no difference in current testosterone regulation in healthy controls compared with non psychotic bipolar women. The difficulty in previous studies looking at testosterone in psychosis has been the confounding effect of medications in women. In males, small studies done prior to the initiation of medications, have shown an oft repeated finding of low free or bioavailable testosterone. This is as far as the author knows the first time it has been possible to study women not currently psychotic and to study the separate effects of psychosis and antipsychotic medications albeit in small numbers of women on testosterone. The doses women were taking were not large compared with those many women take when being treated for schizophrenia but despite this, dramatic testosterone raising effects were seen but really only in a subset of women with a particular androgen receptor polymorphism. In an era where personalized medicine is a goal this is a highly significant interaction which if repeated in larger samples can potentially save many women from severe and problematic side-effects. This is especially so when neuroleptics are gaining ground in a variety of conditions outside of traditional treatment indications and it was specifically those women NOT prone to psychosis by their polymorphism which had the greatest medication induced changes in SHBG. It would thus be of utmost importance to examine the same polymorphism in schizophrenic, depressed and anxious women along with testosterone, SHBG and metabolic indices such as insulin and lipids to see if the relationship holds true in other populations.

Whilst it is still not certain exactly what produces sexually dimorphic patterns in digit length one can still observe that digit length ratios traditionally thought of as masculine (<0.95) was associated with early onset psychosis in this group of bipolar women. Likewise a distinct feminine pattern (>0.98) was associated with late debut of psychotic symptoms. Perhaps this is not surprising given that testosterone has a role during foetal development in cerebral lateralization via for example the corpus callosum, important in inter-hemispheric communication and thus for interpretation of both received and internal stimuli. One could hypothesise that the women with a masculinised D2:D4 ratio would have altered brain asymmetry than women at the other extreme, a question that could be answered with the use of structural MRI. It would also be interesting to note whether these women have more severe psychotic symptoms or whether the ratio relates to other symptoms of psychosis such as disorganization given that delusions and hallucinations may reflect a separate pathophysiological process occurring more fronto-temporally. Thus even though it could not be established that there was a link between psychosis per se and the masculinised ratio it does not rule out there being one given that a comprehensive symptom characterisation of psychosis was lacking in the ADE.

In line with testosterone's role in neurodevelopment the polymorphism in the androgen receptor may reflect a greater degree of androgenisation in early development and thus altered lateralization (potentially greater given the extra androgenisation) which may herald increased risk for psychosis development. One published study to date has examined female and male volume differences with respect to cerebral asymmetry in bipolar disorder and did indeed find increased brain asymmetry in female patients compared with controls (Mackay et al., 2010). These findings contrast with those in males where asymmetry was blunted much as is found in males with schizophrenia, but less severe (Bilder et al., 1999).

5.3 Methodological Considerations:

Subject Selection:

The bipolar patients were recruited predominantly during the years 2005 to 2007 and efforts were made to include both new and existing patients, in other words even well known patients were interviewed according to the above protocol. This means that the patient group is largely representative of those patients attending this specialist outpatient clinic. Also, despite the clinic also serving migrant rich areas at the inception of the study, very few persons of non-northern European background attended the clinic and were thus under-represented in the study. This limits in one sense the generalisability of the results but being advantageous for the genetics part of the study. The inclusion of all patients and not just new referrals meant a large age range of patients. The advantage of this approach is that one can examine longitudinal factors such as for example the number of manic episodes in combination with age on various parameters, but the disadvantage is that it for example prevented identification of parents for the genetics studies, where the inclusion of parental genetic data would enhance the results in a study as small as this. It also precludes the possibility of long-term follow up in the whole cohort, given that the ambition is to have follow-up periods of up to 20 years.

The recruitment of control subjects was randomized yet one cannot extrapolate to say that those who contacted the study team were fully representative of those who were randomly selected. 30 of the 114 controls described alcohol abuse or dependence in at least 1 first or second degree relative. Some of these controls were subsequently excluded due to their own alcohol history. Yet having a large number of control persons with a familial history of alcohol abuse may in fact be advantageous in teasing out the effects of bipolarity

from substance use disorders when a number of bipolar patients also have comorbid substance use disorders.

Symptom Characterization:

The comprehensive collection of information from multiple sources (patient interview, chart review, and collateral information) ensures good characterization of the sample and reduces the risk of false negatives with regard to symptoms. Rated symptoms were binary in nature with definitions provided of what each symptom referred to. However the absence of a borderline or possible criterion makes it difficult to know if clinicians consistently rated up or down on symptom ratings. Additionally, the lack of structured questions for many items in the ADE make the characterization of symptoms all the more dependent upon clinician experience in rating especially subjective phenomena.

Prior to study start, and during the course of the study, seminars were held at which a researcher familiar with the ADE through the STEP-BD study (ML) instructed and discussed the ADE ratings with the clinicians. Inter-rater reliability of symptom ratings was not deemed feasible in this clinic based study where more than 20 clinicians were involved over time in enrolling patients and interviewing them.

The rating dilemma affected the choice of symptom in paper 3. Depressive psychomotor agitation rather than retardation was chosen as the symptom to examine because agitation is more reliably documented in patient charts than retardation (personal observation). In my experience, retardation is commonly regarded by Swedish clinicians to not only include the observable motor and mental slowing including speech latency that the question refers to but also to speech latency observed in other conditions. This includes hesitancy in answering during an interview, as well as latency in answering due to chaotic thoughts rather than to the absence of or slowed thoughts that occur with psychomotor retardation in major depression. Furthermore, delays in answering and seeming slowing are encountered in dissociative states. The phenomenal understanding is important in teasing out the biological systems involved in their manifestation.

Another area where problems were encountered was in the rating of the concept of psychosis. Even though “psychosis” is rated as regards to diagnostic group in the ADE the protocol makes it impossible to rate individual psychotic symptoms as other than mood related. This meant for example, that clinicians occasionally rated these symptoms as delusions and hallucinations during mania which meant that for example some had been rated as having manic delusions with a bipolar 2 disorder. Furthermore, the lack of rating of thought disorder, bizarreness of delusions and disorganized behaviour as symptom ratings in the ADE results in underestimates of the number of patients deemed psychotic. Additionally, there was sometimes a discrepancy between psychosis ratings and symptom ratings so that ratings stated they had had delusions and or hallucinations but were not rated as psychotic overall. A quality assurance project was thus carried out utilising chart review and, in discussion with co-investigators, the documentation was corrected. It is the corrected data that are used for the analyses in paper 4. The above confusion illustrates the problems inherent in the ADE with respect to psychosis as well as illustrating the problems that ensue when trying to rate phenomenon that are not always retrospectively easy to interpret with regard to mood episode timing and the clinicians wish to specify symptoms where no provision has been made for it. It highlights the need for careful analysis of rating instruments and problem solving at early stages when confusion in ratings appear as well as fine tuning of rating instruments to correct problems. One way of enhancing inter-rater

reliability of threshold scoring of symptoms is having training interviews showing patients with difficulty to rate symptomatology.

Missing Data:

Whilst the ambition was as always to have complete data, the reality was that missing data occurred. This was what enhanced patient participation rates as they did not have to complete all components of the study. A core group of ca 130 patients had complete data (including MR, lumbar puncture, neuropsychological testing) and even here there were variables missing from the ADE for example. Genetic testing was carried out at a particular time point and this meant that not all patients with hormonal data had genetic data and likewise a smaller number of patients had incomplete hormones or hormone data that could not be used because of contraceptive use, or being in luteal phase at sampling point. This, and continued enrolment over time, means that numbers differ between the studies presented here. Statistically this was handled in the first three studies to those with genetic data and then using complete case analysis whereby subjects missing data from at least one variable are excluded in the analysis, a strategy used in 88% of molecular epidemiological studies with missing data (Desai et al., 2011b). There are of course methods for handling missing data, such as multiple imputation of means (Desai et al., 2011a) yet this works best if data are missing at random and in the St Göran bipolar project it is unclear if the patients enrolled at the beginning and later stages really do reflect each other, given the non randomness in for example bipolar 1 and 2 diagnoses across the study period. The decision to exclude patients on the basis of missing data does however mean reduced power in the findings. Given that serum and DNA are biobanked it will be possible to correct this problem later, replicating the study in a much larger cohort given that recruitment to the study is ongoing.

D2:D4 Ratio:

The advantage of using Gruning's method of measuring D2:D4 is that it is robust relying on bone measurements rather than creases which can alter with time in the individual. An added reliability factor is that all the measurements have all been carried out by the same nurse ensuring comparability between measurements. The disadvantage is that very few modern studies have used this measure making our results less directly comparable with them.

Statistical Consideration:

Given the nature of the St. Göran study as a multiarmed and multifaceted study it was not possible to calculate power for each individual study question. Additionally the anticipated differences in hormone concentrations were not known given the lack of previous research in bipolar patients. It is clear that genetic data cannot be adequately powered for in such a small sample if one is to use multiple permutations or correction for the number of tests performed. Whilst a type 1 error cannot be ruled out in such small studies of genetic vulnerability, given the dearth of studies in this field and that the studies are more hypothesis generating than hypothesis testing, it was decided to not correct for multiple testing as this risked creating a type 2 statistical error.

Whilst other hormones are normally distributed in the population free testosterone, bioavailable testosterone and total testosterone have been found to follow a log normal distribution in 161 healthy premenopausal women (Braunstein et al., 2011). It is not known whether it is the addition of postmenopausal women which shifts the distribution to a gamma distribution (which is an extension of a chi square distribution) or whether it is the addition of controls which further skews the distribution.

6 CONCLUSION

The literature shows that neuroactive steroids as well as those classed as neurosteroids by being able to be manufactured in the brain have wide ranging effects in neurodevelopment, in neuroprotection and synaptic sprouting as well as in maintaining balance in the glutamatergic and GABAergic neurotransmitter systems. One can regard them as having tuning roles in maintaining optimal brain functioning.

In the studies presented here it has been shown that euthymic serum concentrations of DHEAS and progesterone are lesser if there has been irritability and paranoia during mood elevation in men only and that men with a history of irritability in mood elevation who had depressive psychomotor agitation showed a higher progesterone than those who had only been irritable. Sex differences have thus been shown, emphasising that extrapolation of data between sexes cannot be done in this field. Furthermore it is suggested that gene variants of those genes coding for steroidogenic enzymes may not only affect hormone concentrations but also contribute to the symptomatology of symptoms regarded as occurring within the mixed spectrum of bipolar disorder.

A polymorphism in the androgen receptor was also linked to testosterone differences in women taking neuroleptics, a finding which has major health implications for women prescribed these substances. The role of testosterone in early neurodevelopment is further emphasised by the correlation between D2:D4 finger length in women and age of onset of psychotic symptoms in bipolar disorder, as well as by the elevated risk of developing psychosis (as defined by delusions and hallucinations) in bipolar disorder if one had the variant A allele rs6152, elsewhere established to be associated with clinical hyperandrogenism.

As the studies are small one can not exclude a type 1 error with respect to genetics findings which need to be reproduced in larger studies as well as reverse phenotyped.

7 ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to the patients of St. Göran affective disorders clinic who so generously shared their time and histories making these studies possible. I would also like to thank the volunteers who took time out of their busy schedules to altruistically give so much to the research. I would also like to thank all my patients through the years who have all in their own ways reinforced my sense of a deep rooted connection between psyche and sex hormones. I also thank them for the privilege of wandering with them through good and bad times. Learning is always a two way street. Without you all I would have been much poorer.

I especially also thank my supervisors. Mikael Landén - an energy bomb – enthusiast, a doer and an organizer as well as being someone who made sure I could articulate my arguments cogently and that the articles were logical and readable. Pernilla Nikamo for being *so* available, easy to work with and who was able to explain things in a way I understood. Martin Schalling for very clear explanations and help with formulations as well as for his enthusiasm about what I was doing. Bryan Mowry – my first hero psychiatrist, who also believed in me, stimulated me in starting the research and showed me that psychiatry was indeed the interface between biology, philosophy and caring. I hope I have been able to continue that tradition in my own work. My sincere thanks also go to my mentor Lisa Ekselius who could come up with down to earth suggestions when I needed them and who took time out of her busy schedule to meet.

Special thanks go to Peter Nordström whose support of my application to the research school for psychiatrists despite me being a long-time, hardened clinician made this thesis possible. Deep gratitude also goes to Kaj Forslund, who gave me the opportunity to do this research, even when it wasn't specifically in the same psychiatric service sector as I was working within. Your regard and humanity has helped many a time... Thank you also to Rosario Leopardi who has shown understanding of the time it takes to do research and who has facilitated this thesis.

Appreciation also goes to Prof Assen Jablensky, a source of inspiration for psychiatric scholarship during my years in the desert... and who along with Tim Lambert and Bryan Mowry taught me the importance of phenomenology and symptom dissection.

I would also like to thank the many superb persons helping making these studies possible: the staff of St. Göran affective disorders clinic, study staff: Sr Martina Wennberg, Sr Agneta Carlswärd-Kjellin as well as Haydeh Olofsson whose easy going natures and support have been invaluable.

And to all my colleagues at Rättpsykiatri Vård Stockholm for putting up with my absences, and my demands on you all: without that this work would not have been possible. A great big hug to you all! There are a number of other important influences from Psykiatri Nordväst – too many to mention. You have by your scholarship provided me with a sense of academic belonging from which I have had the confidence to start this journey.

Affection and thanks also go to several important people in my life: to Ann who has been a real friend and tower of strength, to Ewa who has always believed in me and what I was doing, to Anna Lena who has always been a true friend, to Marie for interesting and fruitful discussions, to Jenny and my mates in Australia whose friendships I value.

Lastly, but not least, I would like to posthumously thank my parents who always encouraged education along with creativity and hard work. They always believed I would turn out a PhD dissertation but would not live to quite see the final product.

8 REFERENCES

- Agartz, I., Sedvall, G.C., Terenius, L., Kulle, B., Frigessi, A., Hall, H., Jonsson, E.G., 2006. BDNF gene variants and brain morphology in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 141B, 513-523.
- Agis-Balboa, R.C., Pinna, G., Pibiri, F., Kadriu, B., Costa, E., Guidotti, A., 2007. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *Proceedings of the National Academy of Sciences of the United States of America* 104, 18736-18741.
- Agis-Balboa, R.C., Pinna, G., Zhubi, A., Maloku, E., Veldic, M., Costa, E., Guidotti, A., 2006. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* 103, 14602-14607.
- Ahn, J., Schumacher, F.R., Berndt, S.I., Pfeiffer, R., Albanes, D., Andriole, G.L., Ardanaz, E., Boeing, H., Bueno-de-Mesquita, B., Chanock, S.J., Clavel-Chapelon, F., Diver, W.R., Feigelson, H.S., Gaziano, J.M., Giovannucci, E., Haiman, C.A., Henderson, B.E., Hoover, R.N., Kolonel, L.N., Kraft, P., Ma, J., Le Marchand, L., Overvad, K., Palli, D., Stattin, P., Stampfer, M., Stram, D.O., Thomas, G., Thun, M.J., Travis, R.C., Trichopoulos, D., Virtamo, J., Weinstein, S.J., Yeager, M., Kaaks, R., Hunter, D.J., Hayes, R.B., 2009. Quantitative trait loci predicting circulating sex steroid hormones in men from the NCI-Breast and Prostate Cancer Cohort Consortium (BPC3). *Human molecular genetics* 18, 3749-3757.
- Akdeniz, F., Taneli, F., Noyan, A., Yuncu, Z., Vahip, S., 2003. Valproate-associated reproductive and metabolic abnormalities: are epileptic women at greater risk than bipolar women? *Progress in neuro-psychopharmacology & biological psychiatry* 27, 115-121.
- Akhondzadeh, S., Rezaei, F., Larijani, B., Nejatisafa, A.A., Kashani, L., Abbasi, S.H., 2006. Correlation between testosterone, gonadotropins and prolactin and severity of negative symptoms in male patients with chronic schizophrenia. *Schizophrenia research* 84, 405-410.
- Akimova, E., Lanzenberger, R., Kasper, S., 2009. The serotonin-1A receptor in anxiety disorders. *Biological psychiatry* 66, 627-635.
- Akiskal, H., 2002. Classification, Diagnosis and Boundaries of Bipolar Disorders: A Review. In: Maj, M., Akiskal, H., Lopez-Ibor, J.J., Sartorius, N. (Ed.), *Bipolar Disorder*. John Wiley & sons, Chichester.
- Akiskal, H.S., Benazzi, F., 2003. Family history validation of the bipolar nature of depressive mixed states. *Journal of affective disorders* 73, 113-122.
- Akiskal, H.S., Bourgeois, M.L., Angst, J., Post, R., Moller, H., Hirschfeld, R., 2000. Re-evaluating the prevalence of and diagnostic composition within the broad clinical spectrum of bipolar disorders. *Journal of affective disorders* 59 Suppl 1, S5-S30.
- Akiskal, H.S., Hantouche, E.G., Bourgeois, M.L., Azorin, J.M., Sechter, D., Allilaire, J.F., Lancrenon, S., Fraud, J.P., Chatenet-Duchene, L., 1998a. Gender, temperament, and the clinical picture in dysphoric mixed mania: findings from a French national study (EPIMAN). *Journal of affective disorders* 50, 175-186.
- Akiskal, H.S., Placidi, G.F., Maremmi, I., Signoretta, S., Liguori, A., Gervasi, R., Mallya, G., Puzantian, V.R., 1998b. TEMPS-I: delineating the most discriminant traits of the cyclothymic, depressive, hyperthymic and irritable temperaments in a nonpatient population. *Journal of affective disorders* 51, 7-19.
- Akwa, Y., Sananes, N., Guezou, M., Robel, P., Baulieu, E.E., Le Goascogne, C., 1993. Astrocytes and neurosteroids: metabolism of pregnenolone and dehydroepiandrosterone. Regulation by cell density. *The Journal of cell biology* 121, 135-143.
- Aleman, A., Bronk, E., Kessels, R.P., Koppeschaar, H.P., van Honk, J., 2004. A single administration of testosterone improves visuospatial ability in young women. *Psychoneuroendocrinology* 29, 612-617.
- Alhaj, H.A., Massey, A.E., McAllister-Williams, R.H., 2006. Effects of DHEA administration on episodic memory, cortisol and mood in healthy young men: a double-blind, placebo-controlled study. *Psychopharmacology* 188, 541-551.
- Allagui, M.S., Hfaiedh, N., Vincent, C., Guermazi, F., Murat, J.C., Croute, F., El Feki, A., 2006. Changes in growth rate and thyroid- and sex-hormones blood levels in rats under sub-chronic lithium treatment. *Human & experimental toxicology* 25, 243-250.
- Alonso, G., Phan, V., Guillemain, I., Saunier, M., Legrand, A., Anol, M., Maurice, T., 2000. Immunocytochemical localization of the sigma(1) receptor in the adult rat central nervous system. *Neuroscience* 97, 155-170.
- Amiaz, R., Seidman, S.N., 2008. Testosterone and depression in men. *Current opinion in endocrinology, diabetes, and obesity* 15, 278-283.
- Andersen, S.L., Thompson, A.P., Krenz, E., Teicher, M.H., 2002. Pubertal changes in gonadal hormones do not underlie adolescent dopamine receptor overproduction. *Psychoneuroendocrinology* 27, 683-691.
- Andreen, L., Nyberg, S., Turkmen, S., van Wingen, G., Fernandez, G., Backstrom, T., 2009. Sex steroid induced negative mood may be explained by the paradoxical effect mediated by GABAA modulators. *Psychoneuroendocrinology* 34, 1121-1132.

- Andreen, L., Sundstrom-Poromaa, I., Bixo, M., Nyberg, S., Backstrom, T., 2006. Allopregnanolone concentration and mood--a bimodal association in postmenopausal women treated with oral progesterone. *Psychopharmacology* 187, 209-221.
- Angst, J., Gamma, A., Benazzi, F., Ajdacic, V., Eich, D., Rossler, W., 2003. Toward a re-definition of subthreshold bipolarity: epidemiology and proposed criteria for bipolar-II, minor bipolar disorders and hypomania. *Journal of affective disorders* 73, 133-146.
- Angst, J., Gamma, A., Benazzi, F., Ajdacic, V., Rossler, W., 2009. Does psychomotor agitation in major depressive episodes indicate bipolarity? Evidence from the Zurich Study. *European archives of psychiatry and clinical neuroscience* 259, 55-63.
- APA, 1968. *Diagnostic and Statistical Manual of Mental disorders - II*. American Psychiatric Association, Washington DC.
- APA, 1980. *DSM-III*. American Psychiatric Association, Washington DC.
- Araghiniknam, M., Chung, S., Nelson-White, T., Eskelson, C., Watson, R.R., 1996. Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans. *Life sciences* 59, PL147-157.
- Aragno, M., Brignardello, E., Tamagno, E., Gatto, V., Danni, O., Boccuzzi, G., 1997. Dehydroepiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. *The Journal of endocrinology* 155, 233-240.
- Arato, M., Frecska, E., Beck, C., An, M., Kiss, H., 2004. Digit length pattern in schizophrenia suggests disturbed prenatal hemispheric lateralization. *Progress in neuro-psychopharmacology & biological psychiatry* 28, 191-194.
- Archer, J., 1991. The influence of testosterone on human aggression. *Br J Psychol* 82 (Pt 1), 1-28.
- Ari, Z., Kutlu, N., Uyanik, B.S., Taneli, F., Buyukyazi, G., Tavli, T., 2004. Serum testosterone, growth hormone, and insulin-like growth factor-1 levels, mental reaction time, and maximal aerobic exercise in sedentary and long-term physically trained elderly males. *The International journal of neuroscience* 114, 623-637.
- Asaba, H., Hosoya, K., Takanaga, H., Ohtsuki, S., Tamura, E., Takizawa, T., Terasaki, T., 2000. Blood-brain barrier is involved in the efflux transport of a neuroactive steroid, dehydroepiandrosterone sulfate, via organic anion transporting polypeptide 2. *Journal of neurochemistry* 75, 1907-1916.
- Association, A.P., 1997. *Structured Clinical Interview for DSM-IV Axis II Personality Disorders*; swedish translation 1998. Pilgrim Press.
- Ataya, K., Mercado, A., Kartaginer, J., Abbasi, A., Moghissi, K.S., 1988. Bone density and reproductive hormones in patients with neuroleptic-induced hyperprolactinemia. *Fertility and sterility* 50, 876-881.
- Aubele, T., Kritzer, M.F., 2011. Androgen Influence on Prefrontal Dopamine Systems in Adult Male Rats: Localization of Cognate Intracellular Receptors in Medial Prefrontal Projections to the Ventral Tegmental Area and Effects of Gonadectomy and Hormone Replacement on Glutamate-Stimulated Extracellular Dopamine Level. *Cereb Cortex*.
- Avgoustinaki, P.D., Mitsopoulou, E., Chlouverakis, G., Triantafillou, T., Venihaki, M., Koukouli, S., Margioris, A.N., 2012. Sex steroids and personality traits in the middle luteal phase of healthy normally menstruating young professional women. *Hormones (Athens, Greece)* 11, 333-343.
- Aydar, E., Palmer, C.P., Klyachko, V.A., Jackson, M.B., 2002. The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. *Neuron* 34, 399-410.
- Azad, N., Pitale, S., Barnes, W.E., Friedman, N., 2003. Testosterone treatment enhances regional brain perfusion in hypogonadal men. *The Journal of clinical endocrinology and metabolism* 88, 3064-3068.
- Azarin, J.M., Akiskal, H., Hantouche, E., 2006. The mood-instability hypothesis in the origin of mood-congruent versus mood-incongruent psychotic distinction in mania: validation in a French National Study of 1090 patients. *Journal of affective disorders* 96, 215-223.
- Azarin, J.M., Kaladjian, A., Adida, M., Fakra, E., Hantouche, E., Lancrenon, S., 2010. Correlates of first-episode polarity in a French cohort of 1089 bipolar I disorder patients: role of temperaments and triggering events. *Journal of affective disorders* 129, 39-46.
- Baischer, W., Koinig, G., Hartmann, B., Huber, J., Langer, G., 1995. Hypothalamic-pituitary-gonadal axis in depressed premenopausal women: elevated blood testosterone concentrations compared to normal controls. *Psychoneuroendocrinology* 20, 553-559.
- Balasubramanian, B., Portillo, W., Reyna, A., Chen, J.Z., Moore, A.N., Dash, P.K., Mani, S.K., 2008a. Nonclassical mechanisms of progesterone action in the brain: I. Protein kinase C activation in the hypothalamus of female rats. *Endocrinology* 149, 5509-5517.
- Balasubramanian, B., Portillo, W., Reyna, A., Chen, J.Z., Moore, A.N., Dash, P.K., Mani, S.K., 2008b. Nonclassical mechanisms of progesterone action in the brain: II. Role of calmodulin-dependent protein kinase II in progesterone-mediated signaling in the hypothalamus of female rats. *Endocrinology* 149, 5518-5526.
- Baldessarini, R.J., Salvatore, P., Khalsa, H.M., Tohen, M., 2010. Dissimilar morbidity following initial mania versus mixed-states in type-I bipolar disorder. *Journal of affective disorders* 126, 299-302.

- Baptista, T., Alastre, T., Contreras, Q., Martinez, J.L., Araujo de Baptista, E., Paez, X., Hernandez, L., 1997. Effects of the antipsychotic drug sulpiride on reproductive hormones in healthy men: relationship with body weight regulation. *Pharmacopsychiatry* 30, 250-255.
- Barbaccia, M.L., Affricano, D., Purdy, R.H., Maciocco, E., Spiga, F., Biggio, G., 2001. Clozapine, but not haloperidol, increases brain concentrations of neuroactive steroids in the rat. *Neuropsychopharmacology* 25, 489-497.
- Barnett, J.H., Huang, J., Perlis, R.H., Young, M.M., Rosenbaum, J.F., Nierenberg, A.A., Sachs, G., Nimgaonkar, V.L., Miklowitz, D.J., Smoller, J.W., 2010. Personality and bipolar disorder: dissecting state and trait associations between mood and personality. *Psychological medicine*, 1-12.
- Bassett, M.H., White, P.C., Rainey, W.E., 2004. A role for the NGFI-B family in adrenal zonation and adrenocortical disease. *Endocrine research* 30, 567-574.
- Bastianetto, S., Ramassamy, C., Poirier, J., Quirion, R., 1999. Dehydroepiandrosterone (DHEA) protects hippocampal cells from oxidative stress-induced damage. *Brain research* 66, 35-41.
- Baulieu, E.E., 1981. Steroid hormones in the brain: several mechanisms? . In: Fuxe, K., Gustafsson, J.A., Wetterberg, L. (Ed.), *Steroid Hormone Regulation of the Brain*. Pergamon Press, Oxford, pp. 3-14.
- Baulieu, E.E., Robel, P., Schumacher, M., 2001. Neurosteroids: beginning of the story. *International review of neurobiology* 46, 1-32.
- Baum, A.E., Akula, N., Cabanero, M., Cardona, I., Corona, W., Klemens, B., Schulze, T.G., Cichon, S., Rietschel, M., Nothen, M.M., Georgi, A., Schumacher, J., Schwarz, M., Abou Jamra, R., Hofels, S., Propping, P., Satagopan, J., Detera-Wadleigh, S.D., Hardy, J., McMahon, F.J., 2008. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Molecular psychiatry* 13, 197-207.
- Baxter, L.C., Sparks, D.L., Johnson, S.C., Lenoski, B., Lopez, J.E., Connor, D.J., Sabbagh, M.N., 2006. Relationship of cognitive measures and gray and white matter in Alzheimer's disease. *J Alzheimers Dis* 9, 253-260.
- Beaulieu, J.M., 2011. A role for Akt and glycogen synthase kinase-3 as integrators of dopamine and serotonin neurotransmission in mental health. *J Psychiatry Neurosci* 36, 110011.
- Beckley, E.H., Scibelli, A.C., Finn, D.A., 2011. Progesterone receptor antagonist CDB-4124 increases depression-like behavior in mice without affecting locomotor ability. *Psychoneuroendocrinology* 36, 824-833.
- Bejerot, S., Eriksson, J.M., Bonde, S., Carlstrom, K., Humble, M.B., Eriksson, E., 2012. The extreme male brain revisited: gender coherence in adults with autism spectrum disorder. *Br J Psychiatry* 201, 116-123.
- Beltran, D., Cavas, M., Navarro, J.F., 2006. Effects of (+)SKF 10047, a sigma-1 selective agonist, on isolation-induced aggression in male mice. *Methods and findings in experimental and clinical pharmacology* 28, 601-604.
- Benazzi, F., 2001. Factor analysis of the Montgomery Asberg Depression Rating Scale in 251 bipolar II and 306 unipolar depressed outpatients. *Progress in neuro-psychopharmacology & biological psychiatry* 25, 1369-1376.
- Benazzi, F., 2005. Family history validation of a definition of mixed depression. *Comprehensive psychiatry* 46, 159-166.
- Benazzi, F., Akiskal, H.S., 2006. Psychometric delineation of the most discriminant symptoms of depressive mixed states. *Psychiatry research* 141, 81-88.
- Benedetti, F., Dallaspezia, S., Colombo, C., Lorenzi, C., Pirovano, A., Smeraldi, E., 2010. Association between catechol-O-methyltransferase Val(108/158)Met polymorphism and psychotic features of bipolar disorder. *Journal of affective disorders* 125, 341-344.
- Bergeron, R., de Montigny, C., Debonnel, G., 1996. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J Neurosci* 16, 1193-1202.
- Bermack, J.E., Debonnel, G., 2005. The role of sigma receptors in depression. *Journal of pharmacological sciences* 97, 317-336.
- Berman, A.H., Bergman, H., Palmstierna, T., Schlyter, F., 2005. Evaluation of the Drug Use Disorders Identification Test (DUDIT) in criminal justice and detoxification settings and in a Swedish population sample. *European addiction research* 11, 22-31.
- Bertschy, G., Gervasoni, N., Favre, S., Liberek, C., Ragama-Pardos, E., Aubry, J.M., Gex-Fabry, M., Dayer, A., 2007. Phenomenology of mixed states: a principal component analysis study. *Bipolar disorders* 9, 907-912.
- Bethea, C.L., 1994. Regulation of progestin receptors in raphe neurons of steroid-treated monkeys. *Neuroendocrinology* 60, 50-61.
- Bezdicikova, M., Molikova, R., Bebarova, L., Kolar, Z., 2007. Distribution of nuclear receptors for steroid hormones in the human brain: a preliminary study. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia* 151, 69-71.

- Bicikova, M., Hampl, R., Hill, M., Ripova, D., Mohr, P., Putz, Z., 2011. Neuro- and immunomodulatory steroids and other biochemical markers in drug-naïve schizophrenia patients and the effect of treatment with atypical antipsychotics. *Neuro endocrinology letters* 32, 141-147.
- Bilder, R.M., Wu, H., Bogerts, B., Ashtari, M., Robinson, D., Woerner, M., Lieberman, J.A., Degreef, G., 1999. Cerebral volume asymmetries in schizophrenia and mood disorders: a quantitative magnetic resonance imaging study. *Int J Psychophysiol* 34, 197-205.
- Birkenaes, A.B., Birkeland, K.I., Friis, S., Opjordsmoen, S., Andreassen, O.A., 2009. Hormonal markers of metabolic dysregulation in patients with severe mental disorders after olanzapine treatment under real-life conditions. *Journal of clinical psychopharmacology* 29, 109-116.
- Birtwistle, J., Hayden, R.E., Khanim, F.L., Green, R.M., Pearce, C., Davies, N.J., Wake, N., Schrewe, H., Ride, J.P., Chipman, J.K., Bunce, C.M., 2009. The aldo-keto reductase AKR1C3 contributes to 7,12-dimethylbenz(a)anthracene-3,4-dihydrodiol mediated oxidative DNA damage in myeloid cells: implications for leukemogenesis. *Mutation research* 662, 67-74.
- Bixo, M., Backstrom, T., Winblad, B., Andersson, A., 1995. Estradiol and testosterone in specific regions of the human female brain in different endocrine states. *The Journal of steroid biochemistry and molecular biology* 55, 297-303.
- Bloom, M.S., Houston, A.S., Mills, J.L., Molloy, C.A., Hediger, M.L., 2010. Finger bone immaturity and 2D:4D ratio measurement error in the assessment of the hyperandrogenic hypothesis for the etiology of autism spectrum disorders. *Physiology & behavior* 100, 221-224.
- Boccuzzi, G., Aragno, M., Seccia, M., Brignardello, E., Tamagno, E., Albano, E., Danni, O., Bellomo, G., 1997. Protective effect of dehydroepiandrosterone against copper-induced lipid peroxidation in the rat. *Free radical biology & medicine* 22, 1289-1294.
- Bologa, L., Sharma, J., Roberts, E., 1987. Dehydroepiandrosterone and its sulfated derivative reduce neuronal death and enhance astrocytic differentiation in brain cell cultures. *Journal of neuroscience research* 17, 225-234.
- Bora, E., Yucel, M., Pantelis, C., 2009. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. *Journal of affective disorders* 113, 1-20.
- Bora, E., Yucel, M., Pantelis, C., Berk, M., 2010. Meta-analytic review of neurocognition in bipolar II disorder. *Acta psychiatrica Scandinavica*.
- Borobia, A.M., Novalbos, J., Guerra-Lopez, P., Lopez-Rodriguez, R., Tabares, B., Rodriguez, V., Abad-Santos, F., Carcas, A.J., 2009. Influence of sex and CYP2D6 genotype on mirtazapine disposition, evaluated in Spanish healthy volunteers. *Pharmacol Res* 59, 393-398.
- Bortolato, M., Devoto, P., Roncada, P., Frau, R., Flore, G., Saba, P., Pistritto, G., Soggiu, A., Pisanu, S., Zappala, A., Ristaldi, M.S., Tattoli, M., Cuomo, V., Marrosu, F., Barbaccia, M.L., 2011. Isolation rearing-induced reduction of brain 5alpha-reductase expression: relevance to dopaminergic impairments. *Neuropharmacology* 60, 1301-1308.
- Bouras, C., Kovari, E., Hof, P.R., Riederer, B.M., Giannakopoulos, P., 2001. Anterior cingulate cortex pathology in schizophrenia and bipolar disorder. *Acta neuropathologica* 102, 373-379.
- Bowman, R.E., MacLusky, N.J., Sarmiento, Y., Frankfurt, M., Gordon, M., Luine, V.N., 2004. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. *Endocrinology* 145, 3778-3787.
- Brambilla, D.J., O'Donnell, A.B., Matsumoto, A.M., McKinlay, J.B., 2007. Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical endocrinology* 67, 853-862.
- Brambilla, F., Biggio, G., Pisu, M.G., Purdy, R.H., Gerra, G., Zaimovich, A., Serra, M., 2004. Plasma concentrations of anxiolytic neurosteroids in men with normal anxiety scores: a correlation analysis. *Neuropsychobiology* 50, 6-9.
- Brambilla, F., Guerrini, A., Guastalla, A., Rovere, C., Riggi, F., 1975. Neuroendocrine effects of haloperidol therapy in chronic schizophrenia. *Psychopharmacologia* 44, 17-22.
- Brambilla, F., Mellado, C., Alciati, A., Pisu, M.G., Purdy, R.H., Zanone, S., Perini, G., Serra, M., Biggio, G., 2005. Plasma concentrations of anxiolytic neuroactive steroids in men with panic disorder. *Psychiatry research* 135, 185-190.
- Brand, J.S., Chan, M.F., Dowsett, M., Folkerd, E., Wareham, N.J., Luben, R.N., van der Schouw, Y.T., Khaw, K.T., 2011. Cigarette smoking and endogenous sex hormones in postmenopausal women. *The Journal of clinical endocrinology and metabolism* 96, 3184-3192.
- Braunstein, G.D., Reitz, R.E., Buch, A., Schnell, D., Caulfield, M.P., 2011. Testosterone reference ranges in normally cycling healthy premenopausal women. *The journal of sexual medicine* 8, 2924-2934.

- Braverman, E.R., Chen, T.J., Chen, A.L., Kerner, M.M., Tung, H., Waite, R.L., Schoolfield, J., Blum, K., 2009. Preliminary investigation of plasma levels of sex hormones and human growth factor(s), and P300 latency as correlates to cognitive decline as a function of gender. *BMC research notes* 2, 126.
- Breier, A., Buchanan, R.W., 1992. The effects of metabolic stress on plasma progesterone in healthy volunteers and schizophrenic patients. *Life sciences* 51, 1527-1534.
- Brieger, P., Ehrt, U., Marneros, A., 2003. Frequency of comorbid personality disorders in bipolar and unipolar affective disorders. *Comprehensive psychiatry* 44, 28-34.
- Brietzke, E., Stertz, L., Fernandes, B.S., Kauer-Sant'anna, M., Mascarenhas, M., Escosteguy Vargas, A., Chies, J.A., Kapczinski, F., 2009. Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. *Journal of affective disorders* 116, 214-217.
- Brinton, R.D., Thompson, R.F., Foy, M.R., Baudry, M., Wang, J., Finch, C.E., Morgan, T.E., Pike, C.J., Mack, W.J., Stanczyk, F.Z., Nilsen, J., 2008. Progesterone receptors: form and function in brain. *Frontiers in neuroendocrinology* 29, 313-339.
- Brockington, I., Roper, A., 1990. An evaluation of the concept of cycloid psychosis. *Psychopathology* 23, 193-195.
- Brockington, I.F., Leff, J.P., 1979. Schizo-affective psychosis: definitions and incidence. *Psychological medicine* 9, 91-99.
- Bromek, E., Haduch, A., Daniel, W.A., 2010. The ability of cytochrome P450 2D isoforms to synthesize dopamine in the brain: An in vitro study. *European journal of pharmacology* 626, 171-178.
- Brooks, J.H., Reddon, J.R., 1996. Serum testosterone in violent and nonviolent young offenders. *Journal of clinical psychology* 52, 475-483.
- Brophy, M.H., Rush, A.J., Crowley, G., 1983. Cortisol, estradiol, and androgens in acutely ill paranoid schizophrenics. *Biological psychiatry* 18, 583-590.
- Brown, W.A., Laughren, T.P., Williams, B., 1981. Differential effects of neuroleptic agents on the pituitary-gonadal axis in men. *Archives of general psychiatry* 38, 1270-1272.
- Brown, W.M., Hines, M., Fane, B.A., Breedlove, S.M., 2002. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormones and behavior* 42, 380-386.
- Brum, I.S., Spritzer, P.M., Paris, F., Maturana, M.A., Audran, F., Sultan, C., 2005. Association between androgen receptor gene CAG repeat polymorphism and plasma testosterone levels in postmenopausal women. *Journal of the Society for Gynecologic Investigation* 12, 135-141.
- Buzsaki, G., 2006. *Rhythms of the brain*. Oxford University Press, New York.
- Calhoun, V.D., Sui, J., Kiehl, K., Turner, J., Allen, E., Pearson, G., 2012. Exploring the psychosis functional connectome: aberrant intrinsic networks in schizophrenia and bipolar disorder. *Frontiers in psychiatry / Frontiers Research Foundation* 2, 75.
- Camacho-Arroyo, I., Gonzalez-Arenas, A., Espinosa-Raya, J., Pina-Medina, A.G., Picazo, O., 2011. Short- and long-term treatment with estradiol or progesterone modifies the expression of GFAP, MAP2 and Tau in prefrontal cortex and hippocampus. *Life sciences* 89, 123-128.
- Campos, S.B., Miranda, D.M., Souza, B.R., Pereira, P.A., Neves, F.S., Bicalho, M.A., Melillo, P.H., Tramontina, J., Kapczinski, F., Romano-Silva, M.A., Correa, H., 2010a. Association of polymorphisms of the tryptophan hydroxylase 2 gene with risk for bipolar disorder or suicidal behavior. *Journal of psychiatric research* 44, 271-274.
- Campos, S.B., Miranda, D.M., Souza, B.R., Pereira, P.A., Neves, F.S., Tramontina, J., Kapczinski, F., Romano-Silva, M.A., Correa, H., 2010b. Association study of tryptophan hydroxylase 2 gene polymorphisms in bipolar disorder patients with panic disorder comorbidity. *Psychiatric genetics*.
- Canale, D., Caglieresi, C., Moschini, C., Liberati, C.D., Macchia, E., Pinchera, A., Martino, E., 2005. Androgen receptor polymorphism (CAG repeats) and androgenicity. *Clinical endocrinology* 63, 356-361.
- Carlson, G.A., Goodwin, F.K., 1973. The stages of mania. A longitudinal analysis of the manic episode. *Archives of general psychiatry* 28, 221-228.
- Carpenter, W.T., Jr., Fischer, B.A., 2009. Will the Kraepelinian dichotomy survive DSM-V? *Neuropsychopharmacology* 34, 2081-2087.
- Carrier, N., Kabbaj, M., 2012. Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats. *Hormones and behavior* 61, 678-685.
- Cassidy, F., Ahearn, E.P., Carroll, B.J., 2002. Symptom profile consistency in recurrent manic episodes. *Comprehensive psychiatry* 43, 179-181.
- Castaneda, A.E., Tuulio-Henriksson, A., Marttunen, M., Suvisaari, J., Lonnqvist, J., 2008. A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *Journal of affective disorders* 106, 1-27.
- Ceballos, N.A., France, C.R., al'Absi, M., 2007. Influence of naltrexone administration on dehydroepiandrosterone sulfate levels in male and female participants. *Biological psychology* 74, 414-416.

Champagne, J., Lakis, N., Bourque, J., Stip, E., Lipp, O., Mendrek, A., 2011. Progesterone and cerebral function during emotion processing in men and women with schizophrenia. *Schizophrenia Research and Treatment* 2012, 1-6.

Charalampopoulos, I., Tsatsanis, C., Dermitzaki, E., Alexaki, V.I., Castanas, E., Margioris, A.N., Gravanis, A., 2004. Dehydroepiandrosterone and allopregnanolone protect sympathoadrenal medulla cells against apoptosis via antiapoptotic Bcl-2 proteins. *Proceedings of the National Academy of Sciences of the United States of America* 101, 8209-8214.

Charalampopoulos, I., Remboutsika, E., Margioris, A.N., Gravanis, A., 2008. Neurosteroids as modulators of neurogenesis and neuronal survival. *Trends in endocrinology and metabolism: TEM* 19, 300-307.

Chen, S., Wang, J.M., Irwin, R.W., Yao, J., Liu, L., Brinton, R.D., 2011. Allopregnanolone promotes regeneration and reduces beta-amyloid burden in a preclinical model of Alzheimer's disease. *PloS one* 6, e24293.

Cheng, Z.X., Lan, D.M., Wu, P.Y., Zhu, Y.H., Dong, Y., Ma, L., Zheng, P., 2008. Neurosteroid dehydroepiandrosterone sulphate inhibits persistent sodium currents in rat medial prefrontal cortex via activation of sigma-1 receptors. *Experimental neurology* 210, 128-136.

Cherrier, M.M., 2005. Androgens and cognitive function. *Journal of endocrinological investigation* 28, 65-75.

Christian, H.C., Rolls, N.J., Morris, J.F., 2000. Nongenomic actions of testosterone on a subset of lactotrophs in the male rat pituitary. *Endocrinology* 141, 3111-3119.

Chura, L.R., Lombardo, M.V., Ashwin, E., Auyeung, B., Chakrabarti, B., Bullmore, E.T., Baron-Cohen, S., 2010. Organizational effects of fetal testosterone on human corpus callosum size and asymmetry. *Psychoneuroendocrinology* 35, 122-132.

Claeson, L.-E., Esbjörnsson, E., Carlé, B.-M., Wahlbin, M. rev by Nyman, H, 1971. Claeson-Dahls test for learning and memory Hogrefe psykologi förlaget, Stockholm.

Cobos, E.J., Entrena, J.M., Nieto, F.R., Cendan, C.M., Del Pozo, E., 2008. Pharmacology and therapeutic potential of sigma(1) receptor ligands. *Current neuropharmacology* 6, 344-366.

Collinson, S.L., Lim, M., Chaw, J.H., Verma, S., Sim, K., Rapisarda, A., Chong, S.A., 2010. Increased ratio of 2nd to 4th digit (2D:4D) in schizophrenia. *Psychiatry research* 176, 8-12.

Compagnone, N.A., Mellon, S.H., 1998. Dehydroepiandrosterone: a potential signalling molecule for neocortical organization during development. *Proceedings of the National Academy of Sciences of the United States of America* 95, 4678-4683.

Conejo, N.M., Gonzalez-Pardo, H., Cimadevilla, J.M., Arguelles, J.A., Diaz, F., Vallejo-Seco, G., Arias, J.L., 2005. Influence of gonadal steroids on the glial fibrillary acidic protein-immunoreactive astrocyte population in young rat hippocampus. *Journal of neuroscience research* 79, 488-494.

Connell, J.M., Ball, S.G., Inglis, G.C., Beastall, G.H., Davies, D.L., 1984. The effect of low-dose dopamine infusion on anterior pituitary hormone secretion in normal female subjects. *Clin Sci (Lond)* 67, 219-223.

Connor, C.M., Crawford, B.C., Akbarian, S., 2010. White matter neuron alterations in schizophrenia and related disorders. *Int J Dev Neurosci* 29, 325-334.

Cooper, A.J., Finlayson, R., Velamoor, V.R., Magnus, R.V., Cernovsky, Z., 1989. Effects of ECT on prolactin, LH, FSH and testosterone in males with major depressive illness. *Canadian journal of psychiatry* 34, 814-817.

Corpechot, C., Robel, P., Axelson, M., Sjoval, J., Baulieu, E.E., 1981. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proceedings of the National Academy of Sciences of the United States of America* 78, 4704-4707.

Coryell, W., Winokur, G., Shea, T., Maser, J.D., Endicott, J., Akiskal, H.S., 1994. The long-term stability of depressive subtypes. *The American journal of psychiatry* 151, 199-204.

Coughenour, L.L., Barr, B.M., 2001. Use of trifluoroperazine isolates a [(3)H]ifenprodil binding site in rat brain membranes with the pharmacology of the voltage-independent ifenprodil site on N-methyl-D-aspartate receptors containing NR2B subunits. *The Journal of pharmacology and experimental therapeutics* 296, 150-159.

Cousin, P., Calemard-Michel, L., Lejeune, H., Raverot, G., Yessaad, N., Emptoz-Bonneton, A., Morel, Y., Pugeat, M., 2004. Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. *The Journal of clinical endocrinology and metabolism* 89, 917-924.

Coviello, A.D., Zhuang, W.V., Lunetta, K.L., Bhasin, S., Ulloor, J., Zhang, A., Karasik, D., Kiel, D.P., Vasan, R.S., Murabito, J.M., 2011. Circulating testosterone and SHBG concentrations are heritable in women: the Framingham Heart Study. *The Journal of clinical endocrinology and metabolism* 96, E1491-1495.

Crabbe, P., Bogaert, V., De Bacquer, D., Goemaere, S., Zmierzak, H., Kaufman, J.M., 2007. Part of the interindividual variation in serum testosterone levels in healthy men reflects differences in androgen sensitivity and feedback set point: contribution of the androgen receptor polyglutamine tract polymorphism. *The Journal of clinical endocrinology and metabolism* 92, 3604-3610.

- Craddock, N., O'Donovan, M.C., Owen, M.J., 2009. Psychosis genetics: modeling the relationship between schizophrenia, bipolar disorder, and mixed (or "schizoaffective") psychoses. *Schizophrenia bulletin* 35, 482-490.
- Craddock, N., Owen, M.J., 2005. The beginning of the end for the Kraepelinian dichotomy. *Br J Psychiatry* 186, 364-366.
- Daban, C., Martinez-Aran, A., Torrent, C., Tabares-Seisdedos, R., Balanza-Martinez, V., Salazar-Fraile, J., Selva-Vera, G., Vieta, E., 2006. Specificity of cognitive deficits in bipolar disorder versus schizophrenia. A systematic review. *Psychotherapy and psychosomatics* 75, 72-84.
- Dabbs, J.M., Jr., Frady, R.L., Carr, T.S., Besch, N.F., 1987. Saliva testosterone and criminal violence in young adult prison inmates. *Psychosomatic medicine* 49, 174-182.
- Damassa, D.A., Cates, J.M., 1995. Sex hormone-binding globulin and male sexual development. *Neuroscience and biobehavioral reviews* 19, 165-175.
- Darnell, J.E., Jr., 1997. STATs and gene regulation. *Science (New York, N.Y)* 277, 1630-1635.
- Dazzi, L., Spiga, F., Pira, L., Ladu, S., Vacca, G., Rivano, A., Jentsch, J.D., Biggio, G., 2001a. Inhibition of stress- or anxiogenic-drug-induced increases in dopamine release in the rat prefrontal cortex by long-term treatment with antidepressant drugs. *Journal of neurochemistry* 76, 1212-1220.
- Dazzi, L., Vacca, G., Ladu, S., Pisu, M.G., Serra, M., Biggio, G., 2001b. Long-term treatment with antidepressant drugs reduces the sensitivity of cortical cholinergic neurons to the activating actions of stress and the anxiogenic drug FG 7142. *Neuropharmacology* 41, 229-237.
- De Hert, M., Dekker, J.M., Wood, D., Kahl, K.G., Holt, R.I., Moller, H.J., 2009. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). *Eur Psychiatry* 24, 412-424.
- de Souza Silva, M.A., Mattern, C., Topic, B., Buddenberg, T.E., Huston, J.P., 2009. Dopaminergic and serotonergic activity in neostriatum and nucleus accumbens enhanced by intranasal administration of testosterone. *Eur Neuropsychopharmacol* 19, 53-63.
- Dean, C.E., 2000. Prasterone (DHEA) and mania. *The Annals of pharmacotherapy* 34, 1419-1422.
- Delis, D.C., Kaplan, E., Kramer, J.H., 2001. Delis-Kaplan Executive Function System. Pearson Assessments.
- Dell'Osso, L., Akiskal, H.S., Freer, P., Barberi, M., Placidi, G.F., Cassano, G.B., 1993. Psychotic and nonpsychotic bipolar mixed states: comparisons with manic and schizoaffective disorders. *European archives of psychiatry and clinical neuroscience* 243, 75-81.
- Dell'Osso, L., Placidi, G.F., Nassi, R., Freer, P., Cassano, G.B., Akiskal, H.S., 1991. The manic-depressive mixed state: familial, temperamental and psychopathologic characteristics in 108 female inpatients. *European archives of psychiatry and clinical neuroscience* 240, 234-239.
- Derntl, B., Windischberger, C., Robinson, S., Kryspin-Exner, I., Gur, R.C., Moser, E., Habel, U., 2009. Amygdala activity to fear and anger in healthy young males is associated with testosterone. *Psychoneuroendocrinology* 34, 687-693.
- Derogatis, L.R., Rose, L.I., Shulman, L.H., Lazarus, L.A., 1993. Serum androgens and psychopathology in hirsute women. *Journal of psychosomatic obstetrics and gynaecology* 14, 269-282.
- Desai, M., Esserman, D.A., Gammon, M.D., Terry, M.B., 2011a. The use of complete-case and multiple imputation-based analyses in molecular epidemiology studies that assess interaction effects. *Epidemiol Perspect Innov* 8, 5.
- Desai, M., Kubo, J., Esserman, D., Terry, M.B., 2011b. The handling of missing data in molecular epidemiology studies. *Cancer Epidemiol Biomarkers Prev* 20, 1571-1579.
- Desmarais, J.E., Looper, K.J., 2009. Interactions between tamoxifen and antidepressants via cytochrome P450 2D6. *The Journal of clinical psychiatry* 70, 1688-1697.
- Devoto, P., Frau, R., Bini, V., Pillolla, G., Saba, P., Flore, G., Corona, M., Marrosu, F., Bortolato, M., 2011. Inhibition of 5 α -reductase in the nucleus accumbens counters sensorimotor gating deficits induced by dopaminergic activation. *Psychoneuroendocrinology*.
- Dhir, A., Kulkarni, S., 2008. Involvement of sigma (sigma1) receptors in modulating the anti-depressant effect of neurosteroids (dehydroepiandrosterone or pregnenolone) in mouse tail-suspension test. *Journal of psychopharmacology (Oxford, England)* 22, 691-696.
- Dhir, A., Kulkarni, S.K., 2007. Involvement of sigma-1 receptor modulation in the antidepressant action of venlafaxine. *Neuroscience letters* 420, 204-208.
- di Michele, F., Caltagirone, C., Bonaviri, G., Romeo, E., Spalletta, G., 2005. Plasma dehydroepiandrosterone levels are strongly increased in schizophrenia. *Journal of psychiatric research* 39, 267-273.
- Diamond, D.M., 2004. Enhancement of Cognitive and Electrophysiological Measures of Hippocampal Functioning in Rats by a Low, But Not High, Dose of Dehydroepiandrosterone Sulfate (DHEAS). *Nonlinearity in biology, toxicology, medicine* 2, 371-377.

- Diamond, D.M., Branch, B.J., Fleshner, M., 1996. The neurosteroid dehydroepiandrosterone sulfate (DHEAS) enhances hippocampal primed burst, but not long-term, potentiation. *Neuroscience letters* 202, 204-208.
- Diamond, D.M., Fleshner, M., Rose, G.M., 1999. The enhancement of hippocampal primed burst potentiation by dehydroepiandrosterone sulfate (DHEAS) is blocked by psychological stress. *Stress (Amsterdam, Netherlands)* 3, 107-121.
- Diver, M.J., 2006. Analytical and physiological factors affecting the interpretation of serum testosterone concentration in men. *Annals of clinical biochemistry* 43, 3-12.
- Do Rego, J.L., Mensah-Nyagan, G.A., Beaujean, D., Vaudry, D., Sieghart, W., Luu-The, V., Pelletier, G., Vaudry, H., 2000. Gamma-Aminobutyric acid, acting through gamma-aminobutyric acid type A receptors, inhibits the biosynthesis of neurosteroids in the frog hypothalamus. *Proceedings of the National Academy of Sciences of the United States of America* 97, 13925-13930.
- Do Rego, J.L., Seong, J.Y., Burel, D., Leprince, J., Luu-The, V., Tsutsui, K., Tonon, M.C., Pelletier, G., Vaudry, H., 2009. Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Frontiers in neuroendocrinology* 30, 259-301.
- Dodd, S., Kulkarni, J., Berk, L., Ng, F., Fitzgerald, P.B., de Castella, A.R., Folia, S., Folia, K., Montgomery, W., Kelin, K., Smith, M., Brnabic, A., Berk, M., 2010. A prospective study of the impact of subthreshold mixed states on the 24-month clinical outcomes of bipolar I disorder or schizoaffective disorder. *Journal of affective disorders* 124, 22-28.
- DonCarlos, L.L., Garcia-Ovejero, D., Sarkey, S., Garcia-Segura, L.M., Azcoitia, I., 2003. Androgen receptor immunoreactivity in forebrain axons and dendrites in the rat. *Endocrinology* 144, 3632-3638.
- Dong, L.Y., Cheng, Z.X., Fu, Y.M., Wang, Z.M., Zhu, Y.H., Sun, J.L., Dong, Y., Zheng, P., 2007. Neurosteroid dehydroepiandrosterone sulfate enhances spontaneous glutamate release in rat prelimbic cortex through activation of dopamine D1 and sigma-1 receptor. *Neuropharmacology* 52, 966-974.
- Dorgan, J.F., Reichman, M.E., Judd, J.T., Brown, C., Longcope, C., Schatzkin, A., Campbell, W.S., Franz, C., Kahle, L., Taylor, P.R., 1994. The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control* 5, 53-60.
- Drake, E.B., Henderson, V.W., Stanczyk, F.Z., McCleary, C.A., Brown, W.S., Smith, C.A., Rizzo, A.A., Murdock, G.A., Buckwalter, J.G., 2000. Associations between circulating sex steroid hormones and cognition in normal elderly women. *Neurology* 54, 599-603.
- Ducrey, S., Gex-Fabry, M., Dayer, A., Pardos, E.R., Roth, L., Aubry, J.M., Bertschy, G., 2003. A retrospective comparison of inpatients with mixed and pure depression. *Psychopathology* 36, 292-298.
- Edmunds, S.E., Stubbs, A.P., Santos, A.A., Wilkinson, M.L., 1990. Estrogen and androgen regulation of sex hormone binding globulin secretion by a human liver cell line. *The Journal of steroid biochemistry and molecular biology* 37, 733-739.
- El-Ghundi, M., O'Dowd, B.F., George, S.R., 2007. Insights into the role of dopamine receptor systems in learning and memory. *Reviews in the neurosciences* 18, 37-66.
- el-Samahy, M.H., Shaheen, M.A., Saddik, D.E., Abdel-Fattah, N.S., el-Sawi, M.A., Mahran, M.Z., Shehab, A.A., 2009. Evaluation of androgen receptor gene as a candidate gene in female androgenetic alopecia. *International journal of dermatology* 48, 584-587.
- Ellis, J.A., Panagiotopoulos, S., Akdeniz, A., Jerums, G., Harrap, S.B., 2005. Androgenic correlates of genetic variation in the gene encoding 5alpha-reductase type 1. *Journal of human genetics* 50, 534-537.
- Ellis, J.A., Stebbing, M., Harrap, S.B., 1998. Genetic analysis of male pattern baldness and the 5alpha-reductase genes. *The Journal of investigative dermatology* 110, 849-853.
- Elzanaty, S., Giwercman, Y.L., Giwercman, A., 2006. Significant impact of 5alpha-reductase type 2 polymorphisms on sperm concentration and motility. *International journal of andrology* 29, 414-420.
- Eminovic, I., Komel, R., Prezelj, J., Karamahic, J., Gavrankapetanovic, F., Heljic, B., 2005. Gene analysis of steroid 5 alpha-reductase 1 in hyperandrogenic women. *Croatian medical journal* 46, 664-669.
- Engin, E., Treit, D., 2007. The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: the amygdala, medial prefrontal cortex, or hippocampus. *Behavioural pharmacology* 18, 461-470.
- Erinciler, D., Bugay, G., Ertan, T., Eker, E., 2004. Depression and sex hormones in elderly women. *Archives of gerontology and geriatrics* 39, 239-244.
- Espallergues, J., Givalois, L., Tamsamani, J., Laruelle, C., Maurice, T., 2009. The 3beta-hydroxysteroid dehydrogenase inhibitor trilostane shows antidepressant properties in mice. *Psychoneuroendocrinology* 34, 644-659.
- Espallergues, J., Mamiya, T., Vallee, M., Koseki, T., Nabeshima, T., Tamsamani, J., Laruelle, C., Maurice, T., 2012. The antidepressant-like effects of the 3beta-hydroxysteroid dehydrogenase inhibitor trilostane in mice is related to changes in neuroactive steroid and monoamine levels. *Neuropharmacology* 62, 492-502.

- Estrada, M., Espinosa, A., Muller, M., Jaimovich, E., 2003. Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells. *Endocrinology* 144, 3586-3597.
- Estrada, M., Varshney, A., Ehrlich, B.E., 2006. Elevated testosterone induces apoptosis in neuronal cells. *The Journal of biological chemistry* 281, 25492-25501.
- Fabian, T.J., Dew, M.A., Pollock, B.G., Reynolds, C.F., 3rd, Mulsant, B.H., Butters, M.A., Zmuda, M.D., Linares, A.M., Trottni, M., Kroboth, P.D., 2001. Endogenous concentrations of DHEA and DHEA-S decrease with remission of depression in older adults. *Biological psychiatry* 50, 767-774.
- Fang, F., Christian, W.V., Gorman, S.G., Cui, M., Huang, J., Tieu, K., Ballatori, N., 2010. Neurosteroid transport by the organic solute transporter OSTalpha-OSTbeta. *Journal of neurochemistry* 115, 220-233.
- Feher, A., Juhasz, A., Laszlo, A., Kalman, J., Jr., Pakaski, M., Kalman, J., Janka, Z., 2012. Association between a variant of the sigma-1 receptor gene and Alzheimer's disease. *Neuroscience letters* 517, 136-139.
- Fernandes, B.S., Gama, C.S., Cereser, K.M., Yatham, L.N., Fries, G.R., Colpo, G., de Lucena, D., Kunz, M., Gomes, F.A., Kapczinski, F., 2011. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. *Journal of psychiatric research* 45, 995-1004.
- Fernandez-Cancio, M., Audi, L., Andaluz, P., Toran, N., Piro, C., Albisu, M., Gussinye, M., Yeste, D., Clemente, M., Martinez-Mora, J., Blanco, A., Granada, M.L., Marco, M., Ferragut, J., Lopez-Siguero, J.P., Beneyto, M., Carles, C., Carrascosa, A., 2011. SRD5A2 gene mutations and polymorphisms in Spanish 46,XY patients with a disorder of sex differentiation. *International journal of andrology* 34, e526-e535.
- Fernandez-Egea, E., Garcia-Rizo, C., Miller, B., Parellada, E., Justicia, A., Bernardo, M., Kirkpatrick, B., 2011. Testosterone in newly diagnosed, antipsychotic-naïve men with nonaffective psychosis: a test of the accelerated aging hypothesis. *Psychosomatic medicine* 73, 643-647.
- Fernandez-Guasti, A., del Rio Portilla, I.Y., Ugalde, E., Corsi-Cabrera, M., 2003. Diazepam and progesterone produce sexually dimorphic actions on the rat EEG: role of the neonatal sexual differentiation process. *Psychoneuroendocrinology* 28, 85-100.
- Ferreira, M.A., O'Donovan, M.C., Meng, Y.A., Jones, I.R., Ruderfer, D.M., Jones, L., Fan, J., Kirov, G., Perlis, R.H., Green, E.K., Smoller, J.W., Grozeva, D., Stone, J., Nikolov, I., Chambert, K., Hamshere, M.L., Nimgaonkar, V.L., Moskvina, V., Thase, M.E., Caesar, S., Sachs, G.S., Franklin, J., Gordon-Smith, K., Ardlie, K.G., Gabriel, S.B., Fraser, C., Blumenstiel, B., Defelice, M., Breen, G., Gill, M., Morris, D.W., Elkin, A., Muir, W.J., McGhee, K.A., Williamson, R., MacIntyre, D.J., MacLean, A.W., St, C.D., Robinson, M., Van Beck, M., Pereira, A.C., Kandaswamy, R., McQuillin, A., Collier, D.A., Bass, N.J., Young, A.H., Lawrence, J., Ferrier, I.N., Anjorin, A., Farmer, A., Curtis, D., Scolnick, E.M., McGuffin, P., Daly, M.J., Corvin, A.P., Holmans, P.A., Blackwood, D.H., Gurling, H.M., Owen, M.J., Purcell, S.M., Sklar, P., Craddock, N., 2008. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nature genetics* 40, 1056-1058.
- Figuerola, J.D., Malats, N., Garcia-Closas, M., Real, F.X., Silverman, D., Kogevinas, M., Chanock, S., Welch, R., Dosemeci, M., Lan, Q., Tardon, A., Serra, C., Carrato, A., Garcia-Closas, R., Castano-Vinyals, G., Rothman, N., 2008. Bladder cancer risk and genetic variation in AKR1C3 and other metabolizing genes. *Carcinogenesis* 29, 1955-1962.
- Finley, S.K., Kritzer, M.F., 1999. Immunoreactivity for intracellular androgen receptors in identified subpopulations of neurons, astrocytes and oligodendrocytes in primate prefrontal cortex. *Journal of neurobiology* 40, 446-457.
- Fiore, C., Inman, D.M., Hirose, S., Noble, L.J., Igarashi, T., Compagnone, N.A., 2004. Treatment with the neurosteroid dehydroepiandrosterone promotes recovery of motor behavior after moderate contusive spinal cord injury in the mouse. *Journal of neuroscience research* 75, 391-400.
- Fishback, J.A., Robson, M.J., Xu, Y.T., Matsumoto, R.R., 2010. Sigma receptors: potential targets for a new class of antidepressant drug. *Pharmacology & therapeutics* 127, 271-282.
- Fletcher, E.J., Church, J., MacDonald, J.F., 1994. Haloperidol blocks voltage-activated Ca²⁺ channels in hippocampal neurones. *European journal of pharmacology* 267, 249-252.
- Follesa, P., Biggio, F., Caria, S., Gorini, G., Biggio, G., 2004. Modulation of GABA(A) receptor gene expression by allopregnanolone and ethanol. *European journal of pharmacology* 500, 413-425.
- Fontani, G., Lodi, L., Felici, A., Corradeschi, F., Lupo, C., 2004. Attentional, emotional and hormonal data in subjects of different ages. *European journal of applied physiology* 92, 452-461.
- Foradori, C.D., Weiser, M.J., Handa, R.J., 2008. Non-genomic actions of androgens. *Frontiers in neuroendocrinology* 29, 169-181.
- Fornito, A., Yucel, M., Pantelis, C., 2009. Reconciling neuroimaging and neuropathological findings in schizophrenia and bipolar disorder. *Current opinion in psychiatry* 22, 312-319.

- Funk, A.J., McCullumsmith, R.E., Haroutunian, V., Meador-Woodruff, J.H., 2012. Abnormal activity of the MAPK- and cAMP-associated signaling pathways in frontal cortical areas in postmortem brain in schizophrenia. *Neuropsychopharmacology* 37, 896-905.
- Gallagher, P., Watson, S., Smith, M.S., Young, A.H., Ferrier, I.N., 2007. Plasma cortisol-dehydroepiandrosterone (DHEA) ratios in schizophrenia and bipolar disorder. *Schizophrenia research* 90, 258-265.
- Garey, L., 2010. When cortical development goes wrong: schizophrenia as a neurodevelopmental disease of microcircuits. *Journal of anatomy* 217, 324-333.
- Garner, B., Phassoulitis, C., Phillips, L.J., Markulev, C., Butselaar, F., Bendall, S., Yun, Y., McGorry, P.D., 2011. Cortisol and dehydroepiandrosterone-sulphate levels correlate with symptom severity in first-episode psychosis. *Journal of psychiatric research* 45, 249-255.
- Gaudiano, B.A., Uebelacker, L.A., Miller, I.W., 2007. Course of illness in psychotic mania: is mood incongruence important? *The Journal of nervous and mental disease* 195, 226-232.
- Genazzani, A.D., Luisi, M., Malavasi, B., Strucchi, C., Luisi, S., Casarosa, E., Bernardi, F., Genazzani, A.R., Petraglia, F., 2002. Pulsatile secretory characteristics of allopregnanolone, a neuroactive steroid, during the menstrual cycle and in amenorrheic subjects. *European journal of endocrinology / European Federation of Endocrine Societies* 146, 347-356.
- Geng, Y.G., Su, Q.R., Su, L.Y., Chen, Q., Ren, G.Y., Shen, S.Q., Yu, A.Y., Xia, G.Y., 2007. Comparison of the polymorphisms of androgen receptor gene and estrogen alpha and beta gene between adolescent females with first-onset major depressive disorder and controls. *The International journal of neuroscience* 117, 539-547.
- Gingnell, M., Morell, A., Bannbers, E., Wikstrom, J., Sundstrom Poromaa, I., 2012. Menstrual cycle effects on amygdala reactivity to emotional stimulation in premenstrual dysphoric disorder. *Hormones and behavior*.
- Gizerian, S.S., Morrow, A.L., Lieberman, J.A., Grobin, A.C., 2004. Neonatal neurosteroid administration alters parvalbumin expression and neuron number in medial dorsal thalamus of adult rats. *Brain Res* 1012, 66-74.
- Goes, F.S., Zandi, P.P., Miao, K., McMahon, F.J., Steele, J., Willour, V.L., Mackinnon, D.F., Mondimore, F.M., Schweizer, B., Nurnberger, J.I., Jr., Rice, J.P., Scheftner, W., Coryell, W., Berrettini, W.H., Kelsoe, J.R., Byerley, W., Murphy, D.L., Gershon, E.S., Bipolar Disorder Phenome, G., Depaulo, J.R., Jr., McInnis, M.G., Potash, J.B., 2007. Mood-incongruent psychotic features in bipolar disorder: familial aggregation and suggestive linkage to 2p11-q14 and 13q21-33. *The American journal of psychiatry* 164, 236-247.
- Goldberg, D.P., Andrews, G., Hobbs, M.J., 2009a. Where should bipolar disorder appear in the meta-structure? *Psychological medicine* 39, 2071-2081.
- Goldberg, J.F., Perlis, R.H., Bowden, C.L., Thase, M.E., Miklowitz, D.J., Marangell, L.B., Calabrese, J.R., Nierenberg, A.A., Sachs, G.S., 2009b. Manic symptoms during depressive episodes in 1,380 patients with bipolar disorder: findings from the STEP-BD. *The American journal of psychiatry* 166, 173-181.
- Goncharova, N.D., Marenin, V.Y., Oganyan, T.E., 2010. Aging of the hypothalamic-pituitary-adrenal axis in nonhuman primates with depression-like and aggressive behavior. *Aging* 2, 854-866.
- Goodarzi, M.O., Shah, N.A., Antoine, H.J., Pall, M., Guo, X., Azziz, R., 2006. Variants in the 5alpha-reductase type 1 and type 2 genes are associated with polycystic ovary syndrome and the severity of hirsutism in affected women. *The Journal of clinical endocrinology and metabolism* 91, 4085-4091.
- Goodyer, I.M., Herbert, J., Tamplin, A., Altham, P.M., 2000. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *Br J Psychiatry* 177, 499-504.
- Goyal, R.O., Sagar, R., Ammini, A.C., Khurana, M.L., Alias, A.G., 2004. Negative correlation between negative symptoms of schizophrenia and testosterone levels. *Annals of the New York Academy of Sciences* 1032, 291-294.
- Graupp, M., Wehr, E., Schweighofer, N., Pieber, T.R., Obermayer-Pietsch, B., 2011. Association of genetic variants in the two isoforms of 5alpha-reductase, SRD5A1 and SRD5A2, in lean patients with polycystic ovary syndrome. *European journal of obstetrics, gynecology, and reproductive biology* 157, 175-179.
- Grossmann, M., 2011. Low testosterone in men with type 2 diabetes: significance and treatment. *The Journal of clinical endocrinology and metabolism* 96, 2341-2353.
- Gruber, A.J., Pope, H.G., Jr., 2000. Psychiatric and medical effects of anabolic-androgenic steroid use in women. *Psychotherapy and psychosomatics* 69, 19-26.
- Gualtieri, C.T., Morgan, D.W., 2008. The frequency of cognitive impairment in patients with anxiety, depression, and bipolar disorder: an unaccounted source of variance in clinical trials. *The Journal of clinical psychiatry* 69, 1122-1130.
- Gubba, E.M., Fawcett, J.W., Herbert, J., 2004. The effects of corticosterone and dehydroepiandrosterone on neurotrophic factor mRNA expression in primary hippocampal and astrocyte cultures. *Brain research* 127, 48-59.
- Guest, P.C., Schwarz, E., Krishnamurthy, D., Harris, L.W., Leweke, F.M., Rothermundt, M., van Beveren, N.J., Spain, M., Barnes, A., Steiner, J., Rahmoune, H., Bahn, S., 2011. Altered levels of circulating insulin and

other neuroendocrine hormones associated with the onset of schizophrenia. *Psychoneuroendocrinology* 36, 1092-1096.

Gupta, A., Schulze, T.G., Nagarajan, V., Akula, N., Corona, W., Jiang, X.Y., Hunter, N., McMahon, F.J., Detera-Wadleigh, S.D., 2011. Interaction networks of lithium and valproate molecular targets reveal a striking enrichment of apoptosis functional clusters and neurotrophin signaling. *The pharmacogenomics journal*.

Gustavsson, G., Traskman-Bendz, L., Higley, J.D., Westrin, A., 2003. CSF testosterone in 43 male suicide attempters. *Eur Neuropsychopharmacol* 13, 105-109.

Gustavsson, J.P., Bergman, H., Edman, G., Ekselius, L., von Knorring, L., Linder, J., 2000. Swedish universities Scales of Personality (SSP): construction, internal consistency and normative data. *Acta psychiatrica Scandinavica* 102, 217-225.

Ha, S.J., Kim, J.S., Myung, J.W., Lee, H.J., Kim, J.W., 2003. Analysis of genetic polymorphisms of steroid 5alpha-reductase type 1 and 2 genes in Korean men with androgenetic alopecia. *Journal of dermatological science* 31, 135-141.

Hajszan, T., MacLusky, N.J., Leranth, C., 2008. Role of androgens and the androgen receptor in remodeling of spine synapses in limbic brain areas. *Hormones and behavior* 53, 638-646.

Hanner, M., Moebius, F.F., Flandorfer, A., Knaus, H.G., Striessnig, J., Kempner, E., Glossmann, H., 1996. Purification, molecular cloning, and expression of the mammalian sigma1-binding site. *Proceedings of the National Academy of Sciences of the United States of America* 93, 8072-8077.

Hardoy, M.C., Serra, M., Carta, M.G., Contu, P., Pisu, M.G., Biggio, G., 2006. Increased neuroactive steroid concentrations in women with bipolar disorder or major depressive disorder. *Journal of clinical psychopharmacology* 26, 379-384.

Harvey, P.D., Young, K.P., Reichenberg, A., Pogge, D.L., 2009. The factor structure of clinical symptoms in depressed inpatients with unipolar or bipolar spectrum disorders: a preliminary study. *The Journal of nervous and mental disease* 197, 161-165.

Hayashi, T., Maurice, T., Su, T.P., 2000. Ca(2+) signaling via sigma(1)-receptors: novel regulatory mechanism affecting intracellular Ca(2+) concentration. *The Journal of pharmacology and experimental therapeutics* 293, 788-798.

Hayashi, T., Su, T.P., 2007. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* 131, 596-610.

He, X.Y., Wegiel, J., Yang, Y.Z., Pullarkat, R., Schulz, H., Yang, S.Y., 2005. Type 10 17beta-hydroxysteroid dehydrogenase catalyzing the oxidation of steroid modulators of gamma-aminobutyric acid type A receptors. *Molecular and cellular endocrinology* 229, 111-117.

Heggland, S.J., Signs, S.A., Stalvey, J.R., 1997. Testosterone decreases 3beta-hydroxysteroid dehydrogenase-isomerase messenger ribonucleic acid in cultured mouse Leydig cells by a strain-specific mechanism. *Journal of andrology* 18, 646-655.

Hein, R., Abbas, S., Seibold, P., Salazar, R., Flesch-Janys, D., Chang-Claude, J., 2011. Polymorphism Thr160Thr in SRD5A1, involved in the progesterone metabolism, modifies postmenopausal breast cancer risk associated with menopausal hormone therapy. *Breast cancer research and treatment*.

Henry, C., Mitropoulou, V., New, A.S., Koenigsberg, H.W., Silverman, J., Siever, L.J., 2001. Affective instability and impulsivity in borderline personality and bipolar II disorders: similarities and differences. *Journal of psychiatric research* 35, 307-312.

Henry, C., Van den Bulke, D., Bellivier, F., Roy, I., Swendsen, J., M'Bailara, K., Siever, L.J., Leboyer, M., 2008. Affective lability and affect intensity as core dimensions of bipolar disorders during euthymic period. *Psychiatry research* 159, 1-6.

Her, S., Arimochi, H., Morita, K., 2004. Nerve growth factor induces elevation of steroid 5alpha-reductase mRNA levels in rat C6 glioma cells through expression of transcription factor Egr-1. *Brain research* 126, 157-164.

Hermans, E.J., Ramsey, N.F., van Honk, J., 2008. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. *Biological psychiatry* 63, 263-270.

Hickey, M., Doherty, D.A., Hart, R., Norman, R.J., Mattes, E., Atkinson, H.C., Sloboda, D.M., 2010. Maternal and umbilical cord androgen concentrations do not predict digit ratio (2D:4D) in girls: a prospective cohort study. *Psychoneuroendocrinology* 35, 1235-1244.

Higo, S., Hojo, Y., Ishii, H., Komatsuzaki, Y., Ooishi, Y., Murakami, G., Mukai, H., Yamazaki, T., Nakahara, D., Barron, A., Kimoto, T., Kawato, S., 2011. Endogenous synthesis of corticosteroids in the hippocampus. *PloS one* 6, e21631.

Himmelhoch, J.M., Mulla, D., Neil, J.F., Detre, T.P., Kupfer, D.J., 1976. Incidence and significance of mixed affective states in a bipolar population. *Archives of general psychiatry* 33, 1062-1066.

Hirshman, E., Merriitt, P., Wang, C.C., Wierman, M., Budescu, D.V., Kohrt, W., Templin, J.L., Bhasin, S., 2004. Evidence that androgenic and estrogenic metabolites contribute to the effects of dehydroepiandrosterone on cognition in postmenopausal women. *Hormones and behavior* 45, 144-155.

- Ho, H.P., Westberg, L., Annerbrink, K., Olsson, M., Melke, J., Nilsson, S., Baghaei, F., Rosmond, R., Holm, G., Björntorp, P., Andersch, S., Allgulander, C., Eriksson, E., 2004. Association between a functional polymorphism in the progesterone receptor gene and panic disorder in women. *Psychoneuroendocrinology* 29, 1138-1141.
- Hodgkinson, C.A., Goldman, D., Jaeger, J., Persaud, S., Kane, J.M., Lipsky, R.H., Malhotra, A.K., 2004. Disrupted in schizophrenia 1 (DISC1): association with schizophrenia, schizoaffective disorder, and bipolar disorder. *American journal of human genetics* 75, 862-872.
- Hoskins, R.G., Pincus, G., 1949. Sex-hormone relationships in schizophrenic men. *Psychosomatic medicine* 11, 102-109.
- Hovorkova, P., Kristofikova, Z., Horinek, A., Ripova, D., Majer, E., Zach, P., Sellinger, P., Ricny, J., 2008. Lateralization of 17beta-hydroxysteroid dehydrogenase type 10 in hippocampi of demented and psychotic people. *Dementia and geriatric cognitive disorders* 26, 193-198.
- Hsiao, C.C., 2006a. Difference in pre- and post-treatment plasma DHEA levels were significantly and positively correlated with difference in pre- and post-treatment Hamilton depression scores following successful therapy for major depression. *Psychoneuroendocrinology* 31, 839-846.
- Hsiao, C.C., 2006b. Positive correlation between anxiety severity and plasma levels of dehydroepiandrosterone sulfate in medication-free patients experiencing a major episode of depression. *Psychiatry and clinical neurosciences* 60, 746-750.
- Huber, T.J., Tettenborn, C., Leifke, E., Emrich, H.M., 2005. Sex hormones in psychotic men. *Psychoneuroendocrinology* 30, 111-114.
- Hunter, R., Christie, J.E., Whalley, L.J., Bennie, J., Carroll, S., Dick, H., Goodwin, G.M., Wilson, H., Fink, G., 1989. Luteinizing hormone responses to luteinizing hormone releasing hormone (LHRH) in acute mania and the effects of lithium on LHRH and thyrotrophin releasing hormone tests in volunteers. *Psychological medicine* 19, 69-77.
- Hwang, J.Y., Duncan, R.S., Madry, C., Singh, M., Koulen, P., 2009. Progesterone potentiates calcium release through IP3 receptors by an Akt-mediated mechanism in hippocampal neurons. *Cell calcium* 45, 233-242.
- Hönekopp, J., Bartholdt, L., Beier, L., Liebert, A., 2007. Second to fourth digit length ratio (2D:4D) and adult sex hormone levels: new data and a meta-analytic review. *Psychoneuroendocrinology* 32, 313-321.
- Ida, Y., Tsujimaru, S., Nakamura, K., Shirao, I., Mukasa, H., Egami, H., Nakazawa, Y., 1992. Effects of acute and repeated alcohol ingestion on hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal functioning in normal males. *Drug and alcohol dependence* 31, 57-64.
- Imamura, M., Prasad, C., 1998. Modulation of GABA-gated chloride ion influx in the brain by dehydroepiandrosterone and its metabolites. *Biochemical and biophysical research communications* 243, 771-775.
- Isgor, C., Sengelaub, D.R., 1998. Prenatal gonadal steroids affect adult spatial behavior, CA1 and CA3 pyramidal cell morphology in rats. *Hormones and behavior* 34, 183-198.
- Ishimoto, H., Jaffe, R.B., 2011. Development and function of the human fetal adrenal cortex: a key component in the fetoplacental unit. *Endocrine reviews* 32, 317-355.
- Izmirli, M., Arian, B., Bayazit, Y., Alptekin, D., 2011. Associations of polymorphisms in HPC2/ELAC2 and SRD5A2 genes with benign prostate hyperplasia in Turkish men. *Asian Pac J Cancer Prev* 12, 731-733.
- Jabben, N., Arts, B., Krabbendam, L., van Os, J., 2009. Investigating the association between neurocognition and psychosis in bipolar disorder: further evidence for the overlap with schizophrenia. *Bipolar disorders* 11, 166-177.
- Jana, K., Samanta, P.K., De, D.K., 2010. Nicotine diminishes testicular gametogenesis, steroidogenesis, and steroidogenic acute regulatory protein expression in adult albino rats: possible influence on pituitary gonadotropins and alteration of testicular antioxidant status. *Toxicol Sci* 116, 647-659.
- Jaspers, K., 1942. *General Psychopathology*. John Hopkins University Press. 1997. , Baltimore.
- Jaworska-Feil, L., Budziszewska, B., Leskiewicz, M., Lason, W., 2000. Effects of some centrally active drugs on the allopregnanolone synthesis in rat brain. *Polish journal of pharmacology* 52, 359-365.
- Jeckel, C.M., Lopes, R.P., Berleze, M.C., Luz, C., Feix, L., Argimon, II, Stein, L.M., Bauer, M.E., 2010. Neuroendocrine and immunological correlates of chronic stress in 'strictly healthy' populations. *Neuroimmunomodulation* 17, 9-18.
- Jogems-Kosterman, B.J., de Knijff, D.W., Kusters, R., van Hoof, J.J., 2007. Basal cortisol and DHEA levels in women with borderline personality disorder. *Journal of psychiatric research* 41, 1019-1026.
- Johansson, A., Mowry, B., 1996. Pilot study of hirsutism in women with psychiatric disorders. *Biological psychiatry* 39, 149-151.
- Johansson, T., Frandberg, P.A., Nyberg, F., Le Greves, P., 2005. Low concentrations of neuroactive steroids alter kinetics of [3H]ifenprodil binding to the NMDA receptor in rat frontal cortex. *British journal of pharmacology* 146, 894-902.

- Judd, L.L., Schettler, P.J., Akiskal, H., Coryell, W., Fawcett, J., Fiedorowicz, J.G., Solomon, D.A., Keller, M.B., 2012. Prevalence and clinical significance of subsyndromal manic symptoms, including irritability and psychomotor agitation, during bipolar major depressive episodes. *Journal of affective disorders* 138, 440-448.
- Jung-Testas, I., Hu, Z.Y., Baulieu, E.E., Robel, P., 1989. Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* 125, 2083-2091.
- Jylhä, P., Mantere, O., Melartin, T., Suominen, K., Vuorilehto, M., Arvilommi, P., Leppamäki, S., Valtonen, H., Ryttsala, H., Isometsä, E., 2010. Differences in neuroticism and extraversion between patients with bipolar I or II and general population subjects or major depressive disorder patients. *Journal of affective disorders* 125, 42-52.
- Jönsson, E.G., von Gertten, C., Gustavsson, J.P., Yuan, Q.P., Lindblad-Toh, K., Forslund, K., Rylander, G., Mattila-Evenden, M., Asberg, M., Schalling, M., 2001. Androgen receptor trinucleotide repeat polymorphism and personality traits. *Psychiatric genetics* 11, 19-23.
- Kanayama, G., Hudson, J.I., Pope, H.G., Jr., 2010. Illicit anabolic-androgenic steroid use. *Hormones and behavior* 58, 111-121.
- Kancheva, R., Hill, M., Novak, Z., Chrastina, J., Kancheva, L., Starka, L., 2011. Neuroactive steroids in periphery and cerebrospinal fluid. *Neuroscience* 191, 22-27.
- Kaneda, Y., Fujii, A., 2000. Effects of chronic neuroleptic administration on the hypothalamo-pituitary-gonadal axis of male schizophrenics. *Progress in neuro-psychopharmacology & biological psychiatry* 24, 251-258.
- Kaneda, Y., Ohmori, T., 2003. Impact of risperidone medication on quality of life and gonadal axis hormones in schizophrenia male patients with acute exacerbation. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)* 6, 247-252.
- Katyare, S.S., Modi, H.R., Patel, M.A., 2006. Dehydroepiandrosterone treatment alters lipid/phospholipid profiles of rat brain and liver mitochondria. *Current neurovascular research* 3, 273-279.
- Kavitharaj, N.K., Vijayammal, P.L., 1999. Nicotine administration induced changes in the gonadal functions in male rats. *Pharmacology* 58, 2-7.
- Kim, B., Joo, Y.H., Kim, S.Y., Lim, J.H., Kim, E.O., 2011. Personality traits and affective morbidity in patients with bipolar I disorder: the five-factor model perspective. *Psychiatry research* 185, 135-140.
- Kim, S.B., Hill, M., Kwak, Y.T., Hampl, R., Jo, D.H., Morfin, R., 2003. Neurosteroids: Cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *The Journal of clinical endocrinology and metabolism* 88, 5199-5206.
- Kimonides, V.G., Khatibi, N.H., Svendsen, C.N., Sofroniew, M.V., Herbert, J., 1998. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America* 95, 1852-1857.
- Kimonides, V.G., Spillantini, M.G., Sofroniew, M.V., Fawcett, J.W., Herbert, J., 1999. Dehydroepiandrosterone antagonizes the neurotoxic effects of corticosterone and translocation of stress-activated protein kinase 3 in hippocampal primary cultures. *Neuroscience* 89, 429-436.
- Kimoto, T., Tsurugizawa, T., Ohta, Y., Makino, J., Tamura, H., Hojo, Y., Takata, N., Kawato, S., 2001. Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: N-methyl-D-aspartate and calcium-dependent synthesis. *Endocrinology* 142, 3578-3589.
- Kindlundh, A.M., Bergström, M., Monazzam, A., Hallberg, M., Blomqvist, G., Langström, B., Nyberg, F., 2002. Dopaminergic effects after chronic treatment with nandrolone visualized in rat brain by positron emission tomography. *Progress in neuro-psychopharmacology & biological psychiatry* 26, 1303-1308.
- Klatzkin, R.R., Morrow, A.L., Light, K.C., Pedersen, C.A., Girdler, S.S., 2006. Histories of depression, allopregnanolone responses to stress, and premenstrual symptoms in women. *Biological psychology* 71, 2-11.
- Knickmeyer, R.C., Woolson, S., Hamer, R.M., Konneker, T., Gilmore, J.H., 2011. 2D:4D ratios in the first 2 years of life: Stability and relation to testosterone exposure and sensitivity. *Hormones and behavior*.
- Knyazev, G.G., 2011. Cross-frequency coupling of brain oscillations: An impact of state anxiety. *Int J Psychophysiol* 80, 236-245.
- Knyazev, G.G., Schutter, D.J., van Honk, J., 2006. Anxious apprehension increases coupling of delta and beta oscillations. *Int J Psychophysiol* 61, 283-287.
- Ko, Y.H., Jung, S.W., Joe, S.H., Lee, C.H., Jung, H.G., Jung, I.K., Kim, S.H., Lee, M.S., 2007. Association between serum testosterone levels and the severity of negative symptoms in male patients with chronic schizophrenia. *Psychoneuroendocrinology* 32, 385-391.
- Ko, Y.H., Lew, Y.M., Jung, S.W., Joe, S.H., Lee, C.H., Jung, H.G., Lee, M.S., 2008. Short-term testosterone augmentation in male schizophrenics: a randomized, double-blind, placebo-controlled trial. *Journal of clinical psychopharmacology* 28, 375-383.

- Kohama, S.G., Freesh, F., Bethea, C.L., 1992. Immunocytochemical colocalization of hypothalamic progesterin receptors and tyrosine hydroxylase in steroid-treated monkeys. *Endocrinology* 131, 509-517.
- Koukopoulos, A., Sani, G., Koukopoulos, A.E., Manfredi, G., Pacchiarotti, I., Girardi, P., 2007. Melancholia agitata and mixed depression. *Acta Psychiatr Scand Suppl*, 50-57.
- Koulen, P., Madry, C., Duncan, R.S., Hwang, J.Y., Nixon, E., McClung, N., Gregg, E.V., Singh, M., 2008. Progesterone potentiates IP(3)-mediated calcium signaling through Akt/PKB. *Cell Physiol Biochem* 21, 161-172.
- Kousteni, S., Bellido, T., Plotkin, L.I., O'Brien, C.A., Bodenner, D.L., Han, L., Han, K., DiGregorio, G.B., Katzenellenbogen, J.A., Katzenellenbogen, B.S., Roberson, P.K., Weinstein, R.S., Jilka, R.L., Manolagas, S.C., 2001. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell* 104, 719-730.
- Kraepelin, E., 1921. Manic-Depressive Insanity and Paranoia. E. & S. Livingstone, Edinburgh.
- Krithivas, K., Yurgalevitch, S.M., Mohr, B.A., Wilcox, C.J., Batter, S.J., Brown, M., Longcope, C., McKinlay, J.B., Kantoff, P.W., 1999. Evidence that the CAG repeat in the androgen receptor gene is associated with the age-related decline in serum androgen levels in men. *The Journal of endocrinology* 162, 137-142.
- Kritz-Silverstein, D., von Muhlen, D., Laughlin, G.A., Bettencourt, R., 2008. Effects of dehydroepiandrosterone supplementation on cognitive function and quality of life: the DHEA and Well-Ness (DAWN) Trial. *Journal of the American Geriatrics Society* 56, 1292-1298.
- Kritzer, M.F., 1997. Selective colocalization of immunoreactivity for intracellular gonadal hormone receptors and tyrosine hydroxylase in the ventral tegmental area, substantia nigra, and retrorubral fields in the rat. *The Journal of comparative neurology* 379, 247-260.
- Krone, N., Arlt, W., 2009. Genetics of congenital adrenal hyperplasia. *Best practice & research* 23, 181-192.
- Kullak-Ublick, G.A., Fisch, T., Oswald, M., Hagenbuch, B., Meier, P.J., Beuers, U., Paumgartner, G., 1998. Dehydroepiandrosterone sulfate (DHEAS): identification of a carrier protein in human liver and brain. *FEBS letters* 424, 173-176.
- Kurata, K., Takebayashi, M., Morinobu, S., Yamawaki, S., 2004. beta-estradiol, dehydroepiandrosterone, and dehydroepiandrosterone sulfate protect against N-methyl-D-aspartate-induced neurotoxicity in rat hippocampal neurons by different mechanisms. *The Journal of pharmacology and experimental therapeutics* 311, 237-245.
- Lanthier, A., Patwardhan, V.V., 1986. Sex steroids and 5-en-3 beta-hydroxysteroids in specific regions of the human brain and cranial nerves. *Journal of steroid biochemistry* 25, 445-449.
- Landenberger, R., Mitterhauser, M., Kranz, G.S., Spindelegger, C., Wadsak, W., Stein, P., Moser, U., Savli, M., Kletter, K., Kasper, S., 2011. Progesterone level predicts serotonin-1a receptor binding in the male human brain. *Neuroendocrinology* 94, 84-88.
- Leonard, K., 1999. Classification of endogenous psychoses and their differentiated etiology. Springer-Verlag, Wien.
- Lepagnol-Bestel, A.M., Dubertret, C., Benmessaoud, D., Simonneau, M., Ades, J., Kacha, F., Hamdani, N., Gorwood, P., Ramoz, N., 2010. Association of DISC1 gene with schizophrenia in families from two distinct French and Algerian populations. *Psychiatric genetics* 20, 298-303.
- Leranth, C., Petnehazy, O., MacLusky, N.J., 2003. Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *J Neurosci* 23, 1588-1592.
- Leranth, C., Prange-Kiel, J., Frick, K.M., Horvath, T.L., 2004. Low CA1 spine synapse density is further reduced by castration in male non-human primates. *Cereb Cortex* 14, 503-510.
- Lewinsohn, P.M., Seeley, J.R., Buckley, M.E., Klein, D.N., 2002. Bipolar disorder in adolescence and young adulthood. *Child and adolescent psychiatric clinics of North America* 11, 461-475, vii.
- Lewis, N.D.C., Davies, G.R., 1921. A correlative study of endocrine imbalances and mental disease. *The Journal of nervous and mental disease* 54, 385-493.
- Levitt, A.J., Joffe, R.T., 1988. Total and free testosterone in depressed men. *Acta psychiatrica Scandinavica* 77, 346-348.
- Li, Z., Cui, S., Zhang, Z., Zhou, R., Ge, Y., Sokabe, M., Chen, L., 2009. DHEA-neuroprotection and -neurotoxicity after transient cerebral ischemia in rats. *J Cereb Blood Flow Metab* 29, 287-296.
- Lichtenstein, P., Yip, B.H., Bjork, C., Pawitan, Y., Cannon, T.D., Sullivan, P.F., Hultman, C.M., 2009. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet* 373, 234-239.
- Liere, P., Pianos, A., Eychenne, B., Cambourg, A., Liu, S., Griffiths, W., Schumacher, M., Sjoval, J., Baulieu, E.E., 2004. Novel lipoidal derivatives of pregnenolone and dehydroepiandrosterone and absence of their sulfated counterparts in rodent brain. *Journal of lipid research* 45, 2287-2302.
- Lind, A.B., Reis, M., Bengtsson, F., Jonzier-Perey, M., Powell Golay, K., Ahlner, J., Baumann, P., Dahl, M.L., 2009. Steady-state concentrations of mirtazapine, N-desmethyilmirtazapine, 8-hydroxymirtazapine and their enantiomers in relation to cytochrome P450 2D6 genotype, age and smoking behaviour. *Clin Pharmacokinet* 48, 63-70.

- Lindstrom, S., Ma, J., Altshuler, D., Giovannucci, E., Riboli, E., Albanes, D., Allen, N.E., Berndt, S.I., Boeing, H., Bueno-de-Mesquita, H.B., Chanock, S.J., Dunning, A.M., Feigelson, H.S., Gaziano, J.M., Haiman, C.A., Hayes, R.B., Henderson, B.E., Hunter, D.J., Kaaks, R., Kolonel, L.N., Le Marchand, L., Martinez, C., Overvad, K., Siddiq, A., Stampfer, M., Stattin, P., Stram, D.O., Thun, M.J., Trichopoulos, D., Tumino, R., Virtamo, J., Weinstein, S.J., Yeager, M., Kraft, P., Freedman, M.L., 2010. A large study of androgen receptor germline variants and their relation to sex hormone levels and prostate cancer risk. Results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. *The Journal of clinical endocrinology and metabolism* 95, E121-127.
- Liu, C.Y., Hsu, Y.H., Pan, P.C., Wu, M.T., Ho, C.K., Su, L., Xu, X., Li, Y., Christiani, D.C., 2008a. Maternal and offspring genetic variants of AKR1C3 and the risk of childhood leukemia. *Carcinogenesis* 29, 984-990.
- Liu, L., Foroud, T., Xuei, X., Berrettini, W., Byerley, W., Coryell, W., El-Mallakh, R., Gershon, E.S., Kelsoe, J.R., Lawson, W.B., MacKinnon, D.F., McInnis, M., McMahon, F.J., Murphy, D.L., Rice, J., Scheftner, W., Zandi, P.P., Lohoff, F.W., Niculescu, A.B., Meyer, E.T., Edenberg, H.J., Nurnberger, J.I., Jr., 2008b. Evidence of association between brain-derived neurotrophic factor gene and bipolar disorder. *Psychiatric genetics* 18, 267-274.
- Lobo, R.A., Kletzky, O.A., 1983. Normalization of androgen and sex hormone-binding globulin levels after treatment of hyperprolactinemia. *The Journal of clinical endocrinology and metabolism* 56, 562-566.
- Lockhart, E.M., Warner, D.S., Pearlstein, R.D., Penning, D.H., Mehrabani, S., Boustany, R.M., 2002. Allopregnanolone attenuates N-methyl-D-aspartate-induced excitotoxicity and apoptosis in the human NT2 cell line in culture. *Neuroscience letters* 328, 33-36.
- Lord, S.J., Mack, W.J., Van Den Berg, D., Pike, M.C., Ingles, S.A., Haiman, C.A., Wang, W., Parisky, Y.R., Hodis, H.N., Ursin, G., 2005. Polymorphisms in genes involved in estrogen and progesterone metabolism and mammographic density changes in women randomized to postmenopausal hormone therapy: results from a pilot study. *Breast Cancer Res* 7, R336-344.
- Lu, C.W., Lin, T.Y., Wang, C.C., Wang, S.J., 2012. sigma-1 Receptor agonist SKF10047 inhibits glutamate release in rat cerebral cortex nerve endings. *The Journal of pharmacology and experimental therapeutics* 341, 532-542.
- Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., Manning, J.T., 2004. 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early human development* 77, 23-28.
- Luu-The, V., Labrie, F., 2010. The intracrine sex steroid biosynthesis pathways. *Progress in brain research* 181, 177-192.
- Läkemedelsindustriföreningens Service AB, L., 2011. FASS - mirtazapine.
- Maayan, R., Morad, O., Dorfman, P., Overstreet, D.H., Weizman, A., Yadid, G., 2005a. The involvement of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) in blocking the therapeutic effect of electroconvulsive shocks in an animal model of depression. *Eur Neuropsychopharmacol* 15, 253-262.
- Maayan, R., Shaltiel, G., Poyurovsky, M., Ramadan, E., Morad, O., Nechmad, A., Weizman, A., Agam, G., 2004. Chronic lithium treatment affects rat brain and serum dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEA-S) levels. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)* 7, 71-75.
- Maayan, R., Touati-Werner, D., Ram, E., Galdor, M., Weizman, A., 2005b. Is brain dehydroepiandrosterone synthesis modulated by free radicals in mice? *Neuroscience letters* 377, 130-135.
- Maayan, R., Yagorowski, Y., Grupper, D., Weiss, M., Shtatif, B., Kaoud, M.A., Weizman, A., 2000. Basal plasma dehydroepiandrosterone sulfate level: a possible predictor for response to electroconvulsive therapy in depressed psychotic inpatients. *Biological psychiatry* 48, 693-701.
- Maayan, R., Yoran-Hegesh, R., Strous, R., Nechmad, A., Averbuch, E., Weizman, A., Spivak, B., 2003. Three-month treatment course of methylphenidate increases plasma levels of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S) in attention deficit hyperactivity disorder. *Neuropsychobiology* 48, 111-115.
- Mackay, C.E., Roddick, E., Barrick, T.R., Lloyd, A.J., Roberts, N., Crow, T.J., Young, A.H., Ferrier, I.N., 2010. Sex dependence of brain size and shape in bipolar disorder: an exploratory study. *Bipolar disorders* 12, 306-311.
- Maeda, K., Nwulia, E., Chang, J., Balkissoon, R., Ishizuka, K., Chen, H., Zandi, P., McInnis, M.G., Sawa, A., 2006. Differential expression of disrupted-in-schizophrenia (DISC1) in bipolar disorder. *Biological psychiatry* 60, 929-935.
- Magill, C.A., 2004. The boundary between borderline personality disorder and bipolar disorder: current concepts and challenges. *Canadian journal of psychiatry* 49, 551-556.
- Magnusson, K., Meyerson, B.J., 1996. Evidence for regulatory mechanisms maintaining testosterone-dependent AVP concentrations in terminal regions. *Peptides* 17, 263-268.
- Maj, M., Pirozzi, R., Magliano, L., Bartoli, L., 2003. Agitated depression in bipolar I disorder: prevalence, phenomenology, and outcome. *The American journal of psychiatry* 160, 2134-2140.

- Maj, M., Pirozzi, R., Magliano, L., Fiorillo, A., Bartoli, L., 2006. Agitated "unipolar" major depression: prevalence, phenomenology, and outcome. *The Journal of clinical psychiatry* 67, 712-719.
- Majewska, M.D., 1992. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Progress in neurobiology* 38, 379-395.
- Majewska, M.D., Demirgoren, S., Spivak, C.E., London, E.D., 1990. The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the GABAA receptor. *Brain Res* 526, 143-146.
- Mani, S., 2008. Progesterin receptor subtypes in the brain: the known and the unknown. *Endocrinology* 149, 2750-2756.
- Maninger, N., Capitanio, J.P., Mason, W.A., Ruys, J.D., Mendoza, S.P., 2010. Acute and chronic stress increase DHEAS concentrations in rhesus monkeys. *Psychoneuroendocrinology* 35, 1055-1062.
- Maninger, N., Wolkowitz, O.M., Reus, V.I., Epel, E.S., Mellon, S.H., 2009. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Frontiers in neuroendocrinology* 30, 65-91.
- Manning, J.T., Baron-Cohen, S., Wheelwright, S., Sanders, G., 2001. The 2nd to 4th digit ratio and autism. *Developmental medicine and child neurology* 43, 160-164.
- Manning, J.T., Henzi, P., Venkatramana, P., Martin, S., Singh, D., 2003. Second to fourth digit ratio: ethnic differences and family size in English, Indian and South African populations. *Annals of human biology* 30, 579-588.
- Manning, J.T., Scutt, D., Wilson, J., Lewis-Jones, D.I., 1998. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human reproduction (Oxford, England)* 13, 3000-3004.
- Manuck, S.B., Marsland, A.L., Flory, J.D., Gorka, A., Ferrell, R.E., Hariri, A.R., 2010. Salivary testosterone and a trinucleotide (CAG) length polymorphism in the androgen receptor gene predict amygdala reactivity in men. *Psychoneuroendocrinology* 35, 94-104.
- Markianos, M., Panas, M., Kalfakis, N., Vassilopoulos, D., 2007a. Plasma testosterone, dehydroepiandrosterone sulfate, and cortisol in female patients with Huntington's disease. *Neuro endocrinology letters* 28, 199-203.
- Markianos, M., Tripodanakis, J., Sarantidis, D., Hatzimanolis, J., 2007b. Plasma testosterone and dehydroepiandrosterone sulfate in male and female patients with dysthymic disorder. *Journal of affective disorders* 101, 255-258.
- Marneros, A., 2001. Origin and development of concepts of bipolar mixed states. *Journal of affective disorders* 67, 229-240.
- Marron, T.U., Guerini, V., Rusmini, P., Sau, D., Brevini, T.A., Martini, L., Poletti, A., 2005. Androgen-induced neurite outgrowth is mediated by neuritin in motor neurones. *Journal of neurochemistry* 92, 10-20.
- Marx, C.E., Jarskog, L.F., Lauder, J.M., Gilmore, J.H., Lieberman, J.A., Morrow, A.L., 2000. Neurosteroid modulation of embryonic neuronal survival in vitro following anoxia. *Brain Res* 871, 104-112.
- Marx, C.E., VanDoren, M.J., Duncan, G.E., Lieberman, J.A., Morrow, A.L., 2003. Olanzapine and clozapine increase the GABAergic neuroactive steroid allopregnanolone in rodents. *Neuropsychopharmacology* 28, 1-13.
- Mason, J.W., Giller, E.L., Kosten, T.R., 1988. Serum testosterone differences between patients with schizophrenia and those with affective disorder. *Biological psychiatry* 23, 357-366.
- McCartney, J.L., 1929. Dementia praecox as an endocrinopathy with clinical autopsy reports. *Endocrinology* 13, 73-87.
- McElroy, S.L., Strakowski, S.M., Keck, P.E., Jr, Tugrul, K.L., West, S.A., Lonczak, H.S., 1995. Differences and similarities in mixed and pure mania. *Comprehensive psychiatry* 36, 187-194.
- McGrath, C.L., Glatt, S.J., Sklar, P., Le-Niculescu, H., Kuczenski, R., Doyle, A.E., Biederman, J., Mick, E., Faraone, S.V., Niculescu, A.B., Tsuang, M.T., 2009. Evidence for genetic association of RORB with bipolar disorder. *BMC psychiatry* 9, 70.
- McIntyre, R.S., Mancini, D., Eisfeld, B.S., Soczynska, J.K., Grupp, L., Konarski, J.Z., Kennedy, S.H., 2006. Calculated bioavailable testosterone levels and depression in middle-aged men. *Psychoneuroendocrinology* 31, 1029-1035.
- McIntyre, R.S., Mancini, D.A., McCann, S., Srinivasan, J., Kennedy, S.H., 2003. Valproate, bipolar disorder and polycystic ovarian syndrome. *Bipolar disorders* 5, 28-35.
- Meda, S.A., Gill, A., Stevens, M.C., Lorenzoni, R.P., Glahn, D.C., Calhoun, V.D., Sweeney, J.A., Tamminga, C.A., Keshavan, M.S., Thaker, G., Pearlson, G.D., 2012. Differences in Resting-State Functional Magnetic Resonance Imaging Functional Network Connectivity Between Schizophrenia and Psychotic Bipolar Probands and Their Unaffected First-Degree Relatives. *Biological psychiatry*.
- Mehta, P.H., Josephs, R.A., 2010. Testosterone and cortisol jointly regulate dominance: evidence for a dual-hormone hypothesis. *Hormones and behavior* 58, 898-906.
- Melcangi, R.C., Celotti, F., Martini, L., 1994. Progesterone 5-alpha-reduction in neuronal and in different types of glial cell cultures: type 1 and 2 astrocytes and oligodendrocytes. *Brain Res* 639, 202-206.

- Melcangi, R.C., Poletti, A., Cavarretta, I., Celotti, F., Colciago, A., Magnaghi, V., Motta, M., Negri-Cesi, P., Martini, L., 1998. The 5 α -reductase in the central nervous system: expression and modes of control. *The Journal of steroid biochemistry and molecular biology* 65, 295-299.
- Mendrek, A., Lakis, N., Jimenez, J., 2011. Associations of sex steroid hormones with cerebral activations during mental rotation in men and women with schizophrenia. *Psychoneuroendocrinology*.
- Miczek, K.A., Fish, E.W., De Bold, J.F., 2003. Neurosteroids, GABAA receptors, and escalated aggressive behavior. *Hormones and behavior* 44, 242-257.
- Mifsud, A., Choon, A.T., Fang, D., Yong, E.L., 2001. Prostate-specific antigen, testosterone, sex-hormone binding globulin and androgen receptor CAG repeat polymorphisms in subfertile and normal men. *Molecular human reproduction* 7, 1007-1013.
- Miklowitz, D.J., 1992. Longitudinal outcome and medication noncompliance among manic patients with and without mood-incongruent psychotic features. *The Journal of nervous and mental disease* 180, 703-711.
- Milivojevic, V., Kranzler, H.R., Gelernter, J., Burian, L., Covault, J., 2011. Variation in genes encoding the neuroactive steroid synthetic enzymes 5 α -reductase type 1 and 3 α -reductase type 2 is associated with alcohol dependence. *Alcoholism, clinical and experimental research* 35, 946-952.
- Millar, J.K., Wilson-Annan, J.C., Anderson, S., Christie, S., Taylor, M.S., Semple, C.A., Devon, R.S., St Clair, D.M., Muir, W.J., Blackwood, D.H., Porteous, D.J., 2000. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Human molecular genetics* 9, 1415-1423.
- Miller, K.K., Perlis, R.H., Papakostas, G.I., Mischoulon, D., Losifescu, D.V., Brick, D.J., Fava, M., 2009. Low-dose transdermal testosterone augmentation therapy improves depression severity in women. *CNS spectrums* 14, 688-694.
- Milman, A., Zohar, O., Maayan, R., Weizman, R., Pick, C.G., 2008. DHEAS repeated treatment improves cognitive and behavioral deficits after mild traumatic brain injury. *Eur Neuropsychopharmacol* 18, 181-187.
- Milne, E., White, S., Campbell, R., Swettenham, J., Hansen, P., Ramus, F., 2006. Motion and form coherence detection in autistic spectrum disorder: Relationship to motor control and 2:4 digit ratio. *Journal of autism and developmental disorders* 36, 225-237.
- Misra, M., Papakostas, G.I., Klibanski, A., 2004. Effects of psychiatric disorders and psychotropic medications on prolactin and bone metabolism. *The Journal of clinical psychiatry* 65, 1607-1618; quiz 1590, 1760-1601.
- Molitch, M.E., Rebar, R.W., Barsano, C.P., 1993. Effect of human prolactin administration on gonadotropin and thyrotropin secretion in normal men. *Journal of endocrinological investigation* 16, 559-564.
- Mong, J.A., McCarthy, M.M., 1999. Steroid-induced developmental plasticity in hypothalamic astrocytes: implications for synaptic patterning. *Journal of neurobiology* 40, 602-619.
- Monnet, F.P., de Costa, B.R., Bowen, W.D., 1996. Differentiation of sigma ligand-activated receptor subtypes that modulate NMDA-evoked [3H]-noradrenaline release in rat hippocampal slices. *British journal of pharmacology* 119, 65-72.
- Monnet, F.P., Debonnel, G., Junien, J.L., De Montigny, C., 1990. N-methyl-D-aspartate-induced neuronal activation is selectively modulated by sigma receptors. *European journal of pharmacology* 179, 441-445.
- Monteleone, P., Fabrazzo, M., Serra, M., Tortorella, A., Pisu, M.G., Biggio, G., Maj, M., 2004. Long-term treatment with clozapine does not affect morning circulating levels of allopregnanolone and THDOC in patients with schizophrenia: a preliminary study. *Journal of clinical psychopharmacology* 24, 437-440.
- Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. *Br J Psychiatry* 134, 382-389.
- Morel, Y., Tardy, V., 1997. Molecular genetics of 21 hydroxylase deficiency. In: Azziz, R., Nestler, J.E., Dewailly, D. (Ed.), *Androgen Excess Disorders in Women*. Lippincott-Raven, Philadelphia, pp. 159-172.
- Morgan, C.A., 3rd, Rasmuson, A., Pietrzak, R.H., Coric, V., Southwick, S.M., 2009. Relationships among plasma dehydroepiandrosterone and dehydroepiandrosterone sulfate, cortisol, symptoms of dissociation, and objective performance in humans exposed to underwater navigation stress. *Biological psychiatry* 66, 334-340.
- Morgan, C.P., Bale, T.L., 2011. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. *J Neurosci* 31, 11748-11755.
- Morrison, M.F., Freeman, E.W., Lin, H., Sammel, M.D., 2011. Higher DHEA-S (dehydroepiandrosterone sulfate) levels are associated with depressive symptoms during the menopausal transition: results from the PENN Ovarian Aging Study. *Archives of women's mental health* 14, 375-382.
- Mortensen, P.B., Pedersen, C.B., McGrath, J.J., Hougaard, D.M., Norgaard-Petersen, B., Mors, O., Borglum, A.D., Yolken, R.H., 2011. Neonatal antibodies to infectious agents and risk of bipolar disorder: a population-based case-control study. *Bipolar disorders* 13, 624-629.
- Mukai, Y., Higashi, T., Nagura, Y., Shimada, K., 2008. Studies on neurosteroids XXV. Influence of a 5 α -reductase inhibitor, finasteride, on rat brain neurosteroid levels and metabolism. *Biological & pharmaceutical bulletin* 31, 1646-1650.

- Mulchahey, J.J., Ekhtor, N.N., Zhang, H., Kasckow, J.W., Baker, D.G., Geraciotti, T.D., Jr., 2001. Cerebrospinal fluid and plasma testosterone levels in post-traumatic stress disorder and tobacco dependence. *Psychoneuroendocrinology* 26, 273-285.
- Muller, D.C., Giles, G.G., Bassett, J., Morris, H.A., Manning, J.T., Hopper, J.L., English, D.R., Severi, G., 2011. Second to fourth digit ratio (2D:4D) and concentrations of circulating sex hormones in adulthood. *Reprod Biol Endocrinol* 9, 57.
- Multigner, L., Ndong, J.R., Giusti, A., Romana, M., Delacroix-Maillard, H., Cordier, S., Jegou, B., Thome, J.P., Blanchet, P., 2010. Chlordecone exposure and risk of prostate cancer. *J Clin Oncol* 28, 3457-3462.
- Muneoka, K., Kuwagata, M., Shirayama, Y., Ogawa, T., Shioda, S., 2009. Biphasic effects of neonatal allopregnanolone on striatal dopamine metabolism. *Neuroreport* 20, 860-863.
- Munetsuna, E., Hattori, M., Sakimoto, Y., Ishida, A., Sakata, S., Hojo, Y., Kawato, S., Yamazaki, T., 2011. Environmental enrichment alters gene expression of steroidogenic enzymes in the rat hippocampus. *General and comparative endocrinology* 171, 28-32.
- Murphy, B.E., Abbott, F.V., Allison, C.M., Watts, C., Ghadirian, A.M., 2004. Elevated levels of some neuroactive progesterone metabolites, particularly isopregnanolone, in women with chronic fatigue syndrome. *Psychoneuroendocrinology* 29, 245-268.
- Murray, R.M., Sham, P., Van Os, J., Zanelli, J., Cannon, M., McDonald, C., 2004. A developmental model for similarities and dissimilarities between schizophrenia and bipolar disorder. *Schizophrenia research* 71, 405-416.
- Nachshoni, T., Ebert, T., Abramovitch, Y., Assael-Amir, M., Kotler, M., Maayan, R., Weizman, A., Strous, R.D., 2005. Improvement of extrapyramidal symptoms following dehydroepiandrosterone (DHEA) administration in antipsychotic treated schizophrenia patients: a randomized, double-blind placebo controlled trial. *Schizophrenia research* 79, 251-256.
- Nasman, B., Olsson, T., Backstrom, T., Eriksson, S., Grankvist, K., Viitanen, M., Bucht, G., 1991. Serum dehydroepiandrosterone sulfate in Alzheimer's disease and in multi-infarct dementia. *Biological psychiatry* 30, 684-690.
- Navarro, J.F., Beltran, D., Cavas, M., 2012. Effects of (+) SKF 10,047, a sigma-1 receptor agonist, on anxiety, tested in two laboratory models in mice. *Psicothema* 24, 427-430.
- Nechmad, A., Maayan, R., Ramadan, E., Morad, O., Poyurovsky, M., Weizman, A., 2003. Clozapine decreases rat brain dehydroepiandrosterone and dehydroepiandrosterone sulfate levels. *Eur Neuropsychopharmacol* 13, 29-31.
- Nenonen, H., Bjork, C., Skjaerpe, P.A., Giwercman, A., Rylander, L., Svartberg, J., Giwercman, Y.L., 2010. CAG repeat number is not inversely associated with androgen receptor activity in vitro. *Molecular human reproduction* 16, 153-157.
- Neves-Pereira, M., Cheung, J.K., Pasdar, A., Zhang, F., Breen, G., Yates, P., Sinclair, M., Crombie, C., Walker, N., St Clair, D.M., 2005. BDNF gene is a risk factor for schizophrenia in a Scottish population. *Molecular psychiatry* 10, 208-212.
- Neves-Pereira, M., Mundo, E., Muglia, P., King, N., Macciardi, F., Kennedy, J.L., 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *American journal of human genetics* 71, 651-655.
- Nguyen, E.C., McCracken, K.A., Liu, Y., Pouw, B., Matsumoto, R.R., 2005a. Involvement of sigma (sigma) receptors in the acute actions of methamphetamine: receptor binding and behavioral studies. *Neuropharmacology* 49, 638-645.
- Nguyen, P.N., Billiards, S.S., Walker, D.W., Hirst, J.J., 2003. Changes in 5alpha-pregnane steroids and neurosteroidogenic enzyme expression in the perinatal sheep. *Pediatric research* 53, 956-964.
- Nguyen, T.V., Yao, M., Pike, C.J., 2005b. Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. *Journal of neurochemistry* 94, 1639-1651.
- Nicolas, L.B., Pinoteau, W., Papot, S., Routier, S., Guillaumet, G., Mortaud, S., 2001. Aggressive behavior induced by the steroid sulfatase inhibitor COUMATE and by DHEAS in CBA/H mice. *Brain Res* 922, 216-222.
- Nihalani, N.D., Schwartz, T.L., 2007. Mifepristone, a glucocorticoid antagonist for the potential treatment of psychotic major depression. *Curr Opin Investig Drugs* 8, 563-569.
- Niwa, T., Okada, K., Hiroi, T., Imaoka, S., Narimatsu, S., Funae, Y., 2008. Effect of psychotropic drugs on the 21-hydroxylation of neurosteroids, progesterone and allopregnanolone, catalyzed by rat CYP2D4 and human CYP2D6 in the brain. *Biological & pharmaceutical bulletin* 31, 348-351.
- Noda, Y., Kamei, H., Kamei, Y., Nagai, T., Nishida, M., Nabeshima, T., 2000. Neurosteroids ameliorate conditioned fear stress: an association with sigma receptors. *Neuropsychopharmacology* 23, 276-284.
- Noipayak, P., 2009. The ratio of 2nd and 4th digit length in autistic children. *Journal of the Medical Association of Thailand = Chotmaihet thangkaet* 92, 1040-1045.
- Novak, G., Seeman, P., Talerico, T., 2006. Increased expression of calcium/calmodulin-dependent protein kinase IIbeta in frontal cortex in schizophrenia and depression. *Synapse (New York, N.Y)* 59, 61-68.

- Numata, S., Ueno, S., Iga, J., Yamauchi, K., Hongwei, S., Ohta, K., Kinouchi, S., Shibuya-Tayoshi, S., Tayoshi, S., Aono, M., Kameoka, N., Sumitani, S., Tomotake, M., Kaneda, Y., Taniguchi, T., Ishimoto, Y., Ohmori, T., 2006. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. *Neuroscience letters* 401, 1-5.
- Oades, R.D., Schepker, R., 1994. Serum gonadal steroid hormones in young schizophrenic patients. *Psychoneuroendocrinology* 19, 373-385.
- Ohi, K., Hashimoto, R., Yasuda, Y., Fukumoto, M., Yamamori, H., Umeda-Yano, S., Kamino, K., Ikezawa, K., Azechi, M., Iwase, M., Kazui, H., Kasai, K., Takeda, M., 2011. The SIGMAR1 gene is associated with a risk of schizophrenia and activation of the prefrontal cortex. *Progress in neuro-psychopharmacology & biological psychiatry* 35, 1309-1315.
- Ohlsson, C., Labrie, F., Barrett-Connor, E., Karlsson, M.K., Ljunggren, O., Vandenput, L., Mellstrom, D., Tivesten, A., 2010. Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men. *The Journal of clinical endocrinology and metabolism* 95, 4406-4414.
- Ohlsson, C., Wallaschowski, H., Lunetta, K.L., Stolk, L., Perry, J.R., Koster, A., Petersen, A.K., Eriksson, J., Lehtimäki, T., Huhtaniemi, I.T., Hammond, G.L., Maggio, M., Coviello, A.D., Ferrucci, L., Heier, M., Hofman, A., Holliday, K.L., Jansson, J.O., Kahonen, M., Karasik, D., Karlsson, M.K., Kiel, D.P., Liu, Y., Ljunggren, O., Lorentzon, M., Lyytikäinen, L.P., Meitinger, T., Mellstrom, D., Melzer, D., Miljkovic, I., Nauck, M., Nilsson, M., Penninx, B., Pye, S.R., Vasani, R.S., Reincke, M., Rivadeneira, F., Tajar, A., Teumer, A., Uitterlinden, A.G., Ulloor, J., Viikari, J., Volker, U., Volzke, H., Wichmann, H.E., Wu, T.S., Zhuang, W.V., Ziv, E., Wu, F.C., Raitakari, O., Eriksson, A., Bidlingmaier, M., Harris, T.B., Murray, A., de Jong, F.H., Murabito, J.M., Bhasin, S., Vandenput, L., Haring, R., 2011. Genetic determinants of serum testosterone concentrations in men. *PLoS genetics* 7, e1002313.
- Okonmah, A.D., Bradshaw, W.G., Couceyro, P., Soliman, K.F., 1986. The effect of neuroleptic drugs on serum testosterone level in the male rat. *General pharmacology* 17, 235-238.
- Okten, A., Kalyoncu, M., Yaris, N., 2002. The ratio of second- and fourth-digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Early human development* 70, 47-54.
- Olff, M., Guzelcan, Y., de Vries, G.J., Assies, J., Gersons, B.P., 2006. HPA- and HPT-axis alterations in chronic posttraumatic stress disorder. *Psychoneuroendocrinology* 31, 1220-1230.
- Opstad, P.K., 1992. Androgenic hormones during prolonged physical stress, sleep, and energy deficiency. *The Journal of clinical endocrinology and metabolism* 74, 1176-1183.
- Ou, X.M., Chen, K., Shih, J.C., 2006. Glucocorticoid and androgen activation of monoamine oxidase A is regulated differently by R1 and Sp1. *The Journal of biological chemistry* 281, 21512-21525.
- Owen, M.J., Craddock, N., Jablensky, A., 2007. The genetic deconstruction of psychosis. *Schizophrenia bulletin* 33, 905-911.
- Ozcan, M.E., Banoglu, R., 2003. Gonadal hormones in schizophrenia and mood disorders. *European archives of psychiatry and clinical neuroscience* 253, 193-196.
- Ozsoy, S., Esel, E., 2008. Hypothalamic-pituitary-adrenal axis activity, dehydroepiandrosterone sulphate and their relationships with aggression in early and late alcohol withdrawal. *Progress in neuro-psychopharmacology & biological psychiatry* 32, 340-347.
- Ozsoy, S., Esel, E., Hacımusalar, Y., Candan, Z., Kula, M., Turan, T., 2008. [Acute and chronic effects of electroconvulsive therapy on neuroactive steroids in patients with major depressive disorder]. *Türk psikiyatri dergisi = Turkish journal of psychiatry* 19, 341-348.
- Paba, S., Frau, R., Godar, S.C., Devoto, P., Marrosu, F., Bortolato, M., 2011. Steroid 5alpha-reductase as a novel therapeutic target for schizophrenia and other neuropsychiatric disorders. *Current pharmaceutical design* 17, 151-167.
- Pajer, K., Tabbah, R., Gardner, W., Rubin, R.T., Czambel, R.K., Wang, Y., 2006. Adrenal androgen and gonadal hormone levels in adolescent girls with conduct disorder. *Psychoneuroendocrinology* 31, 1245-1256.
- Pal, A., Fontanilla, D., Gopalakrishnan, A., Chae, Y.K., Markley, J.L., Ruoho, A.E., 2012. The sigma-1 receptor protects against cellular oxidative stress and activates antioxidant response elements. *European journal of pharmacology* 682, 12-20.
- Panda, B., Rao, L., Tosh, D., Dixit, H., Padmalatha, V., Kanakavalli, M., Raseswari, T., Deenadayal, M., Gupta, N., Chakrabarty, B., Nallari, P., Singh, L., 2010. Germline study of AR gene of Indian women with ovarian failure. *Gynecol Endocrinol* 27, 1-7.
- Pardridge, W.M., Mietus, L.J., 1979. Transport of steroid hormones through the rat blood-brain barrier. Primary role of albumin-bound hormone. *The Journal of clinical investigation* 64, 145-154.
- Parsons, T.D., Kratz, K.M., Thompson, E., Stanczyk, F.Z., Buckwalter, J.G., 2006. Dhea supplementation and cognition in postmenopausal women. *The International journal of neuroscience* 116, 141-155.
- Patel, M.A., Katyare, S.S., 2006. Treatment with dehydroepiandrosterone (DHEA) stimulates oxidative energy metabolism in the cerebral mitochondria. A comparative study of effects in old and young adult rats. *Neuroscience letters* 402, 131-136.

- Patel, M.A., Katyare, S.S., 2007. Effect of dehydroepiandrosterone (DHEA) treatment on oxidative energy metabolism in rat liver and brain mitochondria. A dose-response study. *Clinical biochemistry* 40, 57-65.
- Pearce, B.D., Kruszon-Moran, D., Jones, J.L., 2012. The Relationship Between Toxoplasma Gondii Infection and Mood Disorders in the Third National Health and Nutrition Survey. *Biological psychiatry*.
- Peng, C.Y., Long, X.Y., Lu, G.X., 2010. Association of AR rs6152G/A gene polymorphism with susceptibility to polycystic ovary syndrome in Chinese women. *Reproduction, fertility, and development* 22, 881-885.
- Penning, T.M., Burczynski, M.E., Jez, J.M., Hung, C.F., Lin, H.K., Ma, H., Moore, M., Palackal, N., Ratnam, K., 2000. Human 3 α -hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *The Biochemical journal* 351, 67-77.
- Penning, T.M., Jin, Y., Heredia, V.V., Lewis, M., 2003. Structure-function relationships in 3 α -hydroxysteroid dehydrogenases: a comparison of the rat and human isoforms. *The Journal of steroid biochemistry and molecular biology* 85, 247-255.
- Penning, T.M., Jin, Y., Steckelbroeck, S., Lanisnik Rizner, T., Lewis, M., 2004. Structure-function of human 3 α -hydroxysteroid dehydrogenases: genes and proteins. *Molecular and cellular endocrinology* 215, 63-72.
- Pereira, A., Fink, G., Sundram, S., 2009. Clozapine-induced ERK1 and ERK2 signaling in prefrontal cortex is mediated by the EGF receptor. *J Mol Neurosci* 39, 185-198.
- Perlis, R.H., Ostacher, M.J., Uher, R., Nierenberg, A.A., Casamassima, F., Kansky, C., Calabrese, J.R., Thase, M., Sachs, G.S., 2009. Stability of symptoms across major depressive episodes in bipolar disorder. *Bipolar disorders* 11, 867-875.
- Perlis, R.H., Purcell, S., Fagerness, J., Kirby, A., Petryshen, T.L., Fan, J., Sklar, P., 2008. Family-based association study of lithium-related and other candidate genes in bipolar disorder. *Archives of general psychiatry* 65, 53-61.
- Perneger, T.V., 1998. What's wrong with Bonferroni adjustments. *BMJ (Clinical research ed)* 316, 1236-1238.
- Perris, C., 1988. The concept of cycloid psychotic disorder. *Psychiatric developments* 6, 37-56.
- Perry, J.R., Weedon, M.N., Langenberg, C., Jackson, A.U., Lyssenko, V., Sparso, T., Thorleifsson, G., Grallert, H., Ferrucci, L., Maggio, M., Paolisso, G., Walker, M., Palmer, C.N., Payne, F., Young, E., Herder, C., Narisu, N., Morken, M.A., Bonnycastle, L.L., Owen, K.R., Shields, B., Knight, B., Bennett, A., Groves, C.J., Ruukonen, A., Jarvelin, M.R., Pearson, E., Pascoe, L., Ferrannini, E., Bornstein, S.R., Stringham, H.M., Scott, L.J., Kuusisto, J., Nilsson, P., Neptin, M., Gjesing, A.P., Pisinger, C., Lauritzen, T., Sandbaek, A., Sampson, M., Zeggini, E., Lindgren, C.M., Steinthorsdottir, V., Thorsteinsdottir, U., Hansen, T., Schwarz, P., Illig, T., Laakso, M., Stefansson, K., Morris, A.D., Groop, L., Pedersen, O., Boehnke, M., Barroso, I., Wareham, N.J., Hattersley, A.T., McCarthy, M.I., Frayling, T.M., 2010. Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. *Human molecular genetics* 19, 535-544.
- Perugi, G., Akiskal, H.S., Micheli, C., Musetti, L., Paiano, A., Quilici, C., Rossi, L., Cassano, G.B., 1997. Clinical subtypes of bipolar mixed states: validating a broader European definition in 143 cases. *Journal of affective disorders* 43, 169-180.
- Peters, M., Mackenzie, K., Bryden, P., 2002. Finger length and distal finger extent patterns in humans. *American journal of physical anthropology* 117, 209-217.
- Pettersson, P., Ellsinger, B.M., Sjoberg, C., Bjorntorp, P., 1990. Fat distribution and steroid hormones in women with alcohol abuse. *Journal of internal medicine* 228, 311-316.
- Piltonen, T., Koivunen, R., Morin-Papunen, L., Ruukonen, A., Huhtaniemi, I.T., Tapanainen, J.S., 2002. Ovarian and adrenal steroid production: regulatory role of LH/HCG. *Human reproduction (Oxford, England)* 17, 620-624.
- Pinna, G., Agis-Balboa, R.C., Pibiri, F., Nelson, M., Guidotti, A., Costa, E., 2008. Neurosteroid biosynthesis regulates sexually dimorphic fear and aggressive behavior in mice. *Neurochemical research* 33, 1990-2007.
- Pinna, G., Costa, E., Guidotti, A., 2006. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. *Psychopharmacology* 186, 362-372.
- Pinna, G., Dong, E., Matsumoto, K., Costa, E., Guidotti, A., 2003. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. *Proceedings of the National Academy of Sciences of the United States of America* 100, 2035-2040.
- Pisu, M.G., Serra, M., 2004. Neurosteroids and neuroactive drugs in mental disorders. *Life sciences* 74, 3181-3197.
- Poletti, A., Coscarella, A., Negri-Cesi, P., Colciago, A., Celotti, F., Martini, L., 1998a. 5 α -reductase isozymes in the central nervous system. *Steroids* 63, 246-251.

- Poletti, A., Negri-Cesi, P., Rabuffetti, M., Colciago, A., Celotti, F., Martini, L., 1998b. Transient expression of the 5 α -reductase type 2 isozyme in the rat brain in late fetal and early postnatal life. *Endocrinology* 139, 2171-2178.
- Pope, H.G., Jr., Katz, D.L., 1988. Affective and psychotic symptoms associated with anabolic steroid use. *The American journal of psychiatry* 145, 487-490.
- Popma, A., Vermeiren, R., Geluk, C.A., Rinne, T., van den Brink, W., Knol, D.L., Jansen, L.M., van Engeland, H., Doreleijers, T.A., 2007. Cortisol moderates the relationship between testosterone and aggression in delinquent male adolescents. *Biological psychiatry* 61, 405-411.
- Porcu, P., Mostallino, M.C., Sogliano, C., Santoru, F., Berretti, R., Concas, A., 2012. Long-term administration with levonorgestrel decreases allopregnanolone levels and alters GABA(A) receptor subunit expression and anxiety-like behavior. *Pharmacology, biochemistry, and behavior* 102, 366-372.
- Postma, A., Meyer, G., Tuiten, A., van Honk, J., Kessels, R.P., Thijssen, J., 2000. Effects of testosterone administration on selective aspects of object-location memory in healthy young women. *Psychoneuroendocrinology* 25, 563-575.
- Pradhan, D.S., Lau, L.Y., Schmidt, K.L., Soma, K.K., 2010. 3 β -HSD in songbird brain: subcellular localization and rapid regulation by estradiol. *Journal of neurochemistry* 115, 667-675.
- Prien, R.F., Himmelhoch, J.M., Kupfer, D.J., 1988. Treatment of mixed mania. *Journal of affective disorders* 15, 9-15.
- Przegalinski, E., Warchol-Kania, A., Budziszewska, B., Jaworska, L., 1987. Effect of repeated administration of antidepressant drugs on the serum and brain concentration of testosterone and its metabolites. *Polish journal of pharmacology and pharmacy* 39, 683-689.
- Puts, D.A., McDaniel, M.A., Jordan, C.L., Breedlove, S.M., 2008. Spatial ability and prenatal androgens: meta-analyses of congenital adrenal hyperplasia and digit ratio (2D:4D) studies. *Archives of sexual behavior* 37, 100-111.
- Rada, R.T., Kellner, R., Winslow, W.W., 1976. Plasma testosterone and aggressive behavior. *Psychosomatics* 17, 138-142.
- Rada, R.T., Laws, D.R., Kellner, R., Stivastava, L., Peake, G., 1983. Plasma androgens in violent and nonviolent sex offenders. *The Bulletin of the American Academy of Psychiatry and the Law* 11, 149-158.
- Rainey, W.E., Nakamura, Y., 2008. Regulation of the adrenal androgen biosynthesis. *The Journal of steroid biochemistry and molecular biology* 108, 281-286.
- Raisinghani, K.H., Dorfman, R.I., Forchielli, E., Gyermek, L., Genther, G., 1968. Uptake of intravenously administered progesterone, pregnanediol and pregnanolone by the rat brain. *Acta endocrinologica* 57, 395-404.
- Rajender, S., Singh, L., Thangaraj, K., 2007. Phenotypic heterogeneity of mutations in androgen receptor gene. *Asian journal of andrology* 9, 147-179.
- Rajkowska, G., Halaris, A., Selemon, L.D., 2001. Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biological psychiatry* 49, 741-752.
- Ramaekers, J.G., Conen, S., de Kam, P.J., Braat, S., Peeters, P., Theunissen, E.L., Ivgy-May, N., 2011. Residual effects of esmirtazapine on actual driving performance: overall findings and an exploratory analysis into the role of CYP2D6 phenotype. *Psychopharmacology* 215, 321-332.
- Rasanen, P., Hakko, H., Visuri, S., Paanila, J., Kapanen, P., Suomela, T., Tiihonen, J., 1999. Serum testosterone levels, mental disorders and criminal behaviour. *Acta psychiatrica Scandinavica* 99, 348-352.
- Rasgon, N.L., Altshuler, L.L., Fairbanks, L., Elman, S., Bitran, J., Labarca, R., Saad, M., Kupka, R., Nolen, W.A., Frye, M.A., Suppes, T., McElroy, S.L., Keck, P.E., Jr., Leverich, G., Grunze, H., Walden, J., Post, R., Mintz, J., 2005a. Reproductive function and risk for PCOS in women treated for bipolar disorder. *Bipolar disorders* 7, 246-259.
- Rasgon, N.L., Reynolds, M.F., Elman, S., Saad, M., Frye, M.A., Bauer, M., Altshuler, L.L., 2005b. Longitudinal evaluation of reproductive function in women treated for bipolar disorder. *Journal of affective disorders* 89, 217-225.
- Rasmusson, A.M., Pinna, G., Paliwal, P., Weisman, D., Gottschalk, C., Charney, D., Krystal, J., Guidotti, A., 2006. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. *Biological psychiatry* 60, 704-713.
- Rattya, J., Pakarinen, A.J., Knip, M., Repo-Outakoski, M., Myllyla, V.V., Isojarvi, J.I., 2001a. Early hormonal changes during valproate or carbamazepine treatment: a 3-month study. *Neurology* 57, 440-444.
- Rattya, J., Turkka, J., Pakarinen, A.J., Knip, M., Kotila, M.A., Lukkarinen, O., Myllyla, V.V., Isojarvi, J.I., 2001b. Reproductive effects of valproate, carbamazepine, and oxcarbazepine in men with epilepsy. *Neurology* 56, 31-36.
- Redoute, J., Stoleru, S., Pugeat, M., Costes, N., Lavenne, F., Le Bars, D., Dechaud, H., Cinotti, L., Pujol, J.F., 2005. Brain processing of visual sexual stimuli in treated and untreated hypogonadal patients. *Psychoneuroendocrinology* 30, 461-482.

- Riechman, S.E., Fabian, T.J., Kroboth, P.D., Ferrell, R.E., 2004. Steroid sulfatase gene variation and DHEA responsiveness to resistance exercise in MERET. *Physiological genomics* 17, 300-306.
- Rietschel, M., Beckmann, L., Strohmaier, J., Georgi, A., Karpushova, A., Schirmbeck, F., Boesshenz, K.V., Schmal, C., Burger, C., Jamra, R.A., Schumacher, J., Hofels, S., Kumsta, R., Entringer, S., Krug, A., Markov, V., Maier, W., Propping, P., Wust, S., Kircher, T., Nothen, M.M., Cichon, S., Schulze, T.G., 2008. G72 and its association with major depression and neuroticism in large population-based groups from Germany. *The American journal of psychiatry* 165, 753-762.
- Rinieris, P., Hatzimanolis, J., Markianos, M., Stefanis, C., 1989. Effects of treatment with various doses of haloperidol on the pituitary-gonadal axis in male schizophrenic patients. *Neuropsychobiology* 22, 146-149.
- Ritsner, M., Gibel, A., Ram, E., Maayan, R., Weizman, A., 2006. Alterations in DHEA metabolism in schizophrenia: two-month case-control study. *Eur Neuropsychopharmacol* 16, 137-146.
- Ritsner, M., Maayan, R., Gibel, A., Strous, R.D., Modai, I., Weizman, A., 2004. Elevation of the cortisol/dehydroepiandrosterone ratio in schizophrenia patients. *Eur Neuropsychopharmacol* 14, 267-273.
- Ritsner, M.S., Strous, R.D., 2010. Neurocognitive deficits in schizophrenia are associated with alterations in blood levels of neurosteroids: a multiple regression analysis of findings from a double-blind, randomized, placebo-controlled, crossover trial with DHEA. *Journal of psychiatric research* 44, 75-80.
- Robichaud, M., Debonnel, G., 2004. Modulation of the firing activity of female dorsal raphe nucleus serotonergic neurons by neuroactive steroids. *The Journal of endocrinology* 182, 11-21.
- Rodeck, C.H., Gill, D., Rosenberg, D.A., Collins, W.P., 1985. Testosterone levels in midtrimester maternal and fetal plasma and amniotic fluid. *Prenatal diagnosis* 5, 175-181.
- Rosano, G.M., 2000. Androgens and coronary artery disease. A sex-specific effect of sex hormones? *European heart journal* 21, 868-871.
- Rosenfeld, R.S., Rosenberg, B.J., Fukushima, D.K., Hellman, L., 1975. 24-Hour secretory pattern of dehydroisoandrosterone and dehydroisoandrosterone sulfate. *The Journal of clinical endocrinology and metabolism* 40, 850-855.
- Rottig, D., Rottig, S., Brieger, P., Marneros, A., 2007. Temperament and personality in bipolar I patients with and without mixed episodes. *Journal of affective disorders* 104, 97-102.
- Rubin, R.T., Poland, R.E., Lesser, I.M., 1989. Neuroendocrine aspects of primary endogenous depression VIII. Pituitary-gonadal axis activity in male patients and matched control subjects. *Psychoneuroendocrinology* 14, 217-229.
- Rupprecht, R., Rupprecht, C., Rupprecht, M., Noder, M., Schwarz, W., 1988. Different reactivity of the hypothalamo-pituitary-gonadal-axis in depression and normal controls. *Pharmacopsychiatry* 21, 438-439.
- Russell, D.W., Wilson, J.D., 1994. Steroid 5 alpha-reductase: two genes/two enzymes. *Annual review of biochemistry* 63, 25-61.
- Sachs, G.S., Thase, M.E., Otto, M.W., Bauer, M., Miklowitz, D., Wisniewski, S.R., Lavori, P., Lebowitz, B., Rudorfer, M., Frank, E., Nierenberg, A.A., Fava, M., Bowden, C., Ketter, T., Marangell, L., Calabrese, J., Kupfer, D., Rosenbaum, J.F., 2003. Rationale, design, and methods of the systematic treatment enhancement program for bipolar disorder (STEP-BD). *Biological psychiatry* 53, 1028-1042.
- Sadock, B., 2005. Signs and Symptoms in Psychiatry. In: Sadock, B.S.a.V. (Ed.), *Comprehensive Textbook of Psychiatry* Lippincott, Williams & Wilkens, Philadelphia, p. 857.
- Sakata, M., Kimura, Y., Naganawa, M., Oda, K., Ishii, K., Chihara, K., Ishiwata, K., 2007. Mapping of human cerebral sigma1 receptors using positron emission tomography and [¹¹C]SA4503. *NeuroImage* 35, 1-8.
- Salvatore, P., Baldessarini, R.J., Tohen, M., Khalsa, H.M., Sanchez-Toledo, J.P., Zarate, C.A., Jr., Vieta, E., Maggini, C., 2011. McLean-Harvard international first-episode project: two-year stability of ICD-10 diagnoses in 500 first-episode psychotic disorder patients. *The Journal of clinical psychiatry*.
- Sanchez, P., Torres, J.M., Olmo, A., O'Valle, F., Ortega, E., 2009. Effects of environmental stress on mRNA and protein expression levels of steroid 5alpha-Reductase isozymes in adult rat brain. *Hormones and behavior* 56, 348-353.
- Sarasin, A., Schlumpf, M., Muller, M., Fleischmann, I., Lauber, M.E., Lichtensteiger, W., 2003. Adrenal-mediated rather than direct effects of nicotine as a basis of altered sex steroid synthesis in fetal and neonatal rat. *Reproductive toxicology* (Elmsford, N.Y) 17, 153-162.
- Sarrieu, A., Dussailant, M., Agid, F., Philibert, D., Agid, Y., Rostene, W., 1986. Autoradiographic localization of glucocorticosteroid and progesterone binding sites in the human post-mortem brain. *Journal of steroid biochemistry* 25, 717-721.
- Sasaki, T., Han, F., Shioda, N., Moriguchi, S., Kasahara, J., Ishiguro, K., Fukunaga, K., 2006. Lithium-induced activation of Akt and CaM kinase II contributes to its neuroprotective action in a rat microsphere embolism model. *Brain Res* 1108, 98-106.
- Sato, T., Bottlender, R., Schroter, A., Moller, H.J., 2003a. Frequency of manic symptoms during a depressive episode and unipolar 'depressive mixed state' as bipolar spectrum. *Acta psychiatrica Scandinavica* 107, 268-274.

- Sato, T., Bottlender, R., Sievas, M., Schroter, A., Hecht, S., Moller, H.J., 2003b. Long-term inter-episode stability of syndromes underlying mania. *Acta psychiatrica Scandinavica* 108, 310-313.
- Sato, T., Bottlender, R., Sievers, M., Schroter, A., Kleindienst, N., Moller, H.J., 2004. Evaluating the inter-episode stability of depressive mixed states. *Journal of affective disorders* 81, 103-113.
- Saunders, J.B., Aasland, O.G., Babor, T.F., de la Fuente, J.R., Grant, M., 1993. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction (Abingdon, England)* 88, 791-804.
- Sawaya, M.E., Shalita, A.R., 1998. Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne. *Journal of cutaneous medicine and surgery* 3, 9-15.
- Schatzl, G., Madersbacher, S., Haitel, A., Gsur, A., Preyer, M., Haidinger, G., Gassner, C., Ochsner, M., Marberger, M., 2003. Associations of serum testosterone with microvessel density, androgen receptor density and androgen receptor gene polymorphism in prostate cancer. *The Journal of urology* 169, 1312-1315.
- Schiavi, R.C., Stimmel, B.B., Mandeli, J., White, D., 1995. Chronic alcoholism and male sexual function. *The American journal of psychiatry* 152, 1045-1051.
- Schijf, C.P., van der Mooren, M.J., Doesburg, W.H., Thomas, C.M., Rolland, R., 1993. Differences in serum lipids, lipoproteins, sex hormone binding globulin and testosterone between the follicular and the luteal phase of the menstrual cycle. *Acta endocrinologica* 129, 130-133.
- Schule, C., Baghai, T.C., Eser, D., Schwarz, M., Bondy, B., Rupprecht, R., 2009. Effects of mirtazapine on dehydroepiandrosterone-sulfate and cortisol plasma concentrations in depressed patients. *Journal of psychiatric research* 43, 538-545.
- Schulze, T.G., 2010. Genetic research into bipolar disorder: the need for a research framework that integrates sophisticated molecular biology and clinically informed phenotype characterization. *The Psychiatric clinics of North America* 33, 67-82.
- Schulze, T.G., McMahon, F.J., 2004. Defining the phenotype in human genetic studies: forward genetics and reverse phenotyping. *Human heredity* 58, 131-138.
- Schulze, T.G., Ohlraun, S., Czerski, P.M., Schumacher, J., Kassem, L., Deschner, M., Gross, M., Tullius, M., Heidmann, V., Kovalenko, S., Jamra, R.A., Becker, T., Leszczynska-Rodziewicz, A., Hauser, J., Illig, T., Klopp, N., Wellek, S., Cichon, S., Henn, F.A., McMahon, F.J., Maier, W., Propping, P., Nothen, M.M., Rietschel, M., 2005. Genotype-phenotype studies in bipolar disorder showing association between the DAOA/G30 locus and persecutory delusions: a first step toward a molecular genetic classification of psychiatric phenotypes. *The American journal of psychiatry* 162, 2101-2108.
- Schutter, D.J., van Honk, J., 2004. Decoupling of midfrontal delta-beta oscillations after testosterone administration. *Int J Psychophysiol* 53, 71-73.
- Schweiger, U., Deuschle, M., Weber, B., Korner, A., Lammers, C.H., Schmider, J., Gotthardt, U., Heuser, I., 1999. Testosterone, gonadotropin, and cortisol secretion in male patients with major depression. *Psychosomatic medicine* 61, 292-296.
- Scott, L.J., Muglia, P., Kong, X.Q., Guan, W., Flickinger, M., Upmanyu, R., Tozzi, F., Li, J.Z., Burmeister, M., Absher, D., Thompson, R.C., Francks, C., Meng, F., Antoniadis, A., Southwick, A.M., Schatzberg, A.F., Bunney, W.E., Barchas, J.D., Jones, E.G., Day, R., Matthews, K., McGuffin, P., Strauss, J.S., Kennedy, J.L., Middleton, L., Roses, A.D., Watson, S.J., Vincent, J.B., Myers, R.M., Farmer, A.E., Akil, H., Burns, D.K., Boehnke, M., 2009. Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry. *Proceedings of the National Academy of Sciences of the United States of America* 106, 7501-7506.
- Segarra, A.C., Strand, F.L., 1989. Perinatal administration of nicotine alters subsequent sexual behavior and testosterone levels of male rats. *Brain Res* 480, 151-159.
- Selemon, L.D., Rajkowska, G., Goldman-Rakic, P.S., 1998. Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *The Journal of comparative neurology* 392, 402-412.
- Selva, D.M., Hammond, G.L., 2009. Peroxisome-proliferator receptor gamma represses hepatic sex hormone-binding globulin expression. *Endocrinology* 150, 2183-2189.
- Selva, D.M., Hogeveen, K.N., Innis, S.M., Hammond, G.L., 2007. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. *The Journal of clinical investigation* 117, 3979-3987.
- Seney, M.L., Walsh, C., Stolakis, R., Sibille, E., 2012. Neonatal testosterone partially organizes sex differences in stress-induced emotionality in mice. *Neurobiology of disease* 46, 486-496.
- Serra, M., Pisul, M.G., Dazzi, L., Purdy, R.H., Biggio, G., 2002. Prevention of the stress-induced increase in the concentration of neuroactive steroids in rat brain by long-term administration of mirtazapine but not of fluoxetine. *Journal of psychopharmacology (Oxford, England)* 16, 133-138.
- Setlur, S.R., Chen, C.X., Hossain, R.R., Ha, J.S., Van Doren, V.E., Stenzel, B., Steiner, E., Oldridge, D., Kitabayashi, N., Banerjee, S., Chen, J.Y., Schafer, G., Horninger, W., Lee, C., Rubin, M.A., Klocker, H.,

Demichelis, F., 2010. Genetic variation of genes involved in dihydrotestosterone metabolism and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 19, 229-239.

Sharshar, T., Bastuji-Garin, S., Polito, A., De Jonghe, B., Stevens, R.D., Maxime, V., Rodriguez, P., Cerf, C., Outin, H., Touraine, P., Laborde, K., 2011. Hormonal status in protracted critical illness and in-hospital mortality. *Critical care (London, England)* 15, R47.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of clinical psychiatry* 59 Suppl 20, 22-33;quiz 34-57.

Sheikha, S.H., Collins, T.J., Rassoli, A.H., LeGate, L.S., Banerji, T.K., 1987. Effects of lithium on the pituitary-gonadal axis in the rat: evidence for dose-dependent changes in plasma gonadotropin and testosterone levels. *Life sciences* 40, 1835-1844.

Shi, J., Wittke-Thompson, J.K., Badner, J.A., Hattori, E., Potash, J.B., Willour, V.L., McMahon, F.J., Gershon, E.S., Liu, C., 2008. Clock genes may influence bipolar disorder susceptibility and dysfunctional circadian rhythm. *Am J Med Genet B Neuropsychiatr Genet* 147B, 1047-1055.

Shih, R.A., Belmonte, P.L., Zandi, P.P., 2004. A review of the evidence from family, twin and adoption studies for a genetic contribution to adult psychiatric disorders. *International review of psychiatry (Abingdon, England)* 16, 260-283.

Shimodaira, M., Nakayama, T., Sato, N., Aoi, N., Sato, M., Izumi, Y., Soma, M., Matsumoto, K., 2010. Association of HSD3B1 and HSD3B2 gene polymorphisms with essential hypertension, aldosterone level, and left ventricular structure. *European journal of endocrinology / European Federation of Endocrine Societies* 163, 671-680.

Shirayama, Y., Hashimoto, K., Suzuki, Y., Higuchi, T., 2002. Correlation of plasma neurosteroid levels to the severity of negative symptoms in male patients with schizophrenia. *Schizophrenia research* 58, 69-74.

Shufelt, C., Merz, C.N., Yang, Y., Kirschner, J., Polk, D., Stanczyk, F., Paul-Labrador, M., Braunstein, G.D., 2011. Red Versus White Wine as a Nutritional Aromatase Inhibitor in Premenopausal Women. *Journal of women's health (2002)*.

Shulman, L.H., DeRogatis, L., Spielvogel, R., Miller, J.L., Rose, L.I., 1992. Serum androgens and depression in women with facial hirsutism. *Journal of the American Academy of Dermatology* 27, 178-181.

Signorelli, S.S., Barresi, V., Musso, N., Anzaldi, M., Croce, E., Fiore, V., Condorelli, D.F., 2008. Polymorphisms of steroid 5-alpha-reductase type I (SRD5A1) gene are associated to peripheral arterial disease. *Journal of endocrinological investigation* 31, 1092-1097.

Silver, H., Knoll, G., Isakov, V., Goodman, C., Finkelstein, Y., 2005. Blood DHEAS concentrations correlate with cognitive function in chronic schizophrenia patients: a pilot study. *Journal of psychiatric research* 39, 569-575.

Simard, J., Ricketts, M.L., Gingras, S., Soucy, P., Feltus, F.A., Melner, M.H., 2005. Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. *Endocrine reviews* 26, 525-582.

Singh, M., 2001. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. *Endocrine* 14, 407-415.

Sklar, P., Gabriel, S.B., McInnis, M.G., Bennett, P., Lim, Y.M., Tsan, G., Schaffner, S., Kirov, G., Jones, I., Owen, M., Craddock, N., DePaulo, J.R., Lander, E.S., 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. *Brain-derived neurotrophic factor. Molecular psychiatry* 7, 579-593.

Sklar, P., Smoller, J.W., Fan, J., Ferreira, M.A., Perlis, R.H., Chambert, K., Nimgaonkar, V.L., McQueen, M.B., Faraone, S.V., Kirby, A., de Bakker, P.I., Ogdie, M.N., Thase, M.E., Sachs, G.S., Todd-Brown, K., Gabriel, S.B., Sougnez, C., Gates, C., Blumenstiel, B., Defelice, M., Ardlie, K.G., Franklin, J., Muir, W.J., McGhee, K.A., MacIntyre, D.J., McLean, A., VanBeck, M., McQuillin, A., Bass, N.J., Robinson, M., Lawrence, J., Anjorin, A., Curtis, D., Scolnick, E.M., Daly, M.J., Blackwood, D.H., Gurling, H.M., Purcell, S.M., 2008. Whole-genome association study of bipolar disorder. *Molecular psychiatry* 13, 558-569.

Smeraldi, E., Benedetti, F., Zanardi, R., 2002. Serotonin transporter promoter genotype and illness recurrence in mood disorders. *Eur Neuropsychopharmacol* 12, 73-75.

Smit, P., van Schaik, R.H., van der Werf, M., van den Beld, A.W., Koper, J.W., Lindemans, J., Pols, H.A., Brinkmann, A.O., de Jong, F.H., Lamberts, S.W., 2005. A common polymorphism in the CYP3A7 gene is associated with a nearly 50% reduction in serum dehydroepiandrosterone sulfate levels. *The Journal of clinical endocrinology and metabolism* 90, 5313-5316.

Smith, E.N., Bloss, C.S., Badner, J.A., Barrett, T., Belmonte, P.L., Berrettini, W., Byerley, W., Coryell, W., Craig, D., Edenberg, H.J., Eskin, E., Foroud, T., Gershon, E., Greenwood, T.A., Hipolito, M., Koller, D.L., Lawson, W.B., Liu, C., Lohoff, F., McInnis, M.G., McMahon, F.J., Mirel, D.B., Murray, S.S., Nievergelt, C., Nurnberger, J., Nwulia, E.A., Paschall, J., Potash, J.B., Rice, J., Schulze, T.G., Scheftner, W., Panganiban, C.,

- Zaitlen, N., Zandi, P.P., Zollner, S., Schork, N.J., Kelsoe, J.R., 2009. Genome-wide association study of bipolar disorder in European American and African American individuals. *Molecular psychiatry* 14, 755-763.
- Smith, L.M., Cloak, C.C., Poland, R.E., Torday, J., Ross, M.G., 2003. Prenatal nicotine increases testosterone levels in the fetus and female offspring. *Nicotine Tob Res* 5, 369-374.
- Snider, N.T., Sikora, M.J., Sridar, C., Feuerstein, T.J., Rae, J.M., Hollenberg, P.F., 2008. The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6. *The Journal of pharmacology and experimental therapeutics* 327, 538-545.
- Soldin, O.P., Makambi, K.H., Soldin, S.J., O'Mara, D.M., 2011. Steroid hormone levels associated with passive and active smoking. *Steroids* 76, 653-659.
- Spalletta, G., Morris, D.W., Angelucci, F., Rubino, I.A., Spoletini, I., Bria, P., Martinotti, G., Siracusano, A., Bonaviri, G., Bernardini, S., Caltagirone, C., Bossu, P., Donohoe, G., Gill, M., Corvin, A.P., 2010. BDNF Val66Met polymorphism is associated with aggressive behavior in schizophrenia. *Eur Psychiatry* 25, 311-313.
- Spalletta, G., Romeo, E., Bonaviri, G., Bernardi, G., Caltagirone, C., di Michele, F., 2005. Preliminary evidence for an association between aggressive and hostile behaviour and 3alpha,5alpha-tetrahydroprogesterone plasma levels in schizophrenia. *J Psychiatry Neurosci* 30, 49-52.
- Spitz, I.M., Bardin, C.W., 1993. Clinical pharmacology of RU 486--an antiprogesterin and antiglucocorticoid. *Contraception* 48, 403-444.
- Spitzer, R.L., Endicott, J., Robins, E., 1975. Clinical criteria for psychiatric diagnosis and DSM-III. *The American journal of psychiatry* 132, 1187-1192.
- Spitzer, R.L., Fleiss, J.L., 1974. A re-analysis of the reliability of psychiatric diagnosis. *Br J Psychiatry* 125, 341-347.
- Spitzer, R.L., Forman, J.B., Nee, J., 1979. DSM-III field trials: I. Initial interrater diagnostic reliability. *The American journal of psychiatry* 136, 815-817.
- Stanton, S.J., Wirth, M.M., Waugh, C.E., Schultheiss, O.C., 2009. Endogenous testosterone levels are associated with amygdala and ventromedial prefrontal cortex responses to anger faces in men but not women. *Biological psychology* 81, 118-122.
- Steckelbroeck, S., Jin, Y., Gopishetty, S., Oyesanmi, B., Penning, T.M., 2004a. Human cytosolic 3alpha-hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3beta-hydroxysteroid dehydrogenase activity: implications for steroid hormone metabolism and action. *The Journal of biological chemistry* 279, 10784-10795.
- Steckelbroeck, S., Nassen, A., Ugele, B., Ludwig, M., Watzka, M., Reissinger, A., Clusmann, H., Lutjohann, D., Siekmann, L., Klingmuller, D., Hans, V.H., 2004b. Steroid sulfatase (STS) expression in the human temporal lobe: enzyme activity, mRNA expression and immunohistochemistry study. *Journal of neurochemistry* 89, 403-417.
- Steckelbroeck, S., Watzka, M., Stoffel-Wagner, B., Hans, V.H., Redel, L., Clusmann, H., Elger, C.E., Bidlingmaier, F., Klingmuller, D., 2001. Expression of the 17beta-hydroxysteroid dehydrogenase type 5 mRNA in the human brain. *Molecular and cellular endocrinology* 171, 165-168.
- Steen, N.E., Tesli, M., Kahler, A.K., Methlie, P., Hope, S., Barrett, E.A., Larsson, S., Mork, E., Lovas, K., Rossberg, J.I., Agartz, I., Melle, I., Djurovic, S., Lorentzen, S., Berg, J.P., Andreassen, O.A., 2010. SRD5A2 is associated with increased cortisol metabolism in schizophrenia spectrum disorders. *Progress in neuro-psychopharmacology & biological psychiatry* 34, 1500-1506.
- Steffensen, S.C., 1995. Dehydroepiandrosterone sulfate suppresses hippocampal recurrent inhibition and synchronizes neuronal activity to theta rhythm. *Hippocampus* 5, 320-328.
- Steffensen, S.C., Jones, M.D., Hales, K., Allison, D.W., 2006. Dehydroepiandrosterone sulfate and estrone sulfate reduce GABA-recurrent inhibition in the hippocampus via muscarinic acetylcholine receptors. *Hippocampus* 16, 1080-1090.
- Steiger, A., von Bardeleben, U., Wiedemann, K., Holsboer, F., 1991. Sleep EEG and nocturnal secretion of testosterone and cortisol in patients with major endogenous depression during acute phase and after remission. *Journal of psychiatric research* 25, 169-177.
- Stephen, L.J., Sills, G.J., Leach, J.P., Butler, E., Parker, P., Hitiris, N., Leach, V.M., Wilson, E.A., Brodie, M.J., 2007. Sodium valproate versus lamotrigine: a randomised comparison of efficacy, tolerability and effects on circulating androgenic hormones in newly diagnosed epilepsy. *Epilepsy research* 75, 122-129.
- Strakowski, S.M., Williams, J.R., Sax, K.W., Fleck, D.E., DelBello, M.P., Bourne, M.L., 2000. Is impaired outcome following a first manic episode due to mood-incongruent psychosis? *Journal of affective disorders* 61, 87-94.
- Strauss, E.B., Sands, D.E., Robinson, A.M., Tindall, W.J., Stevenson, W.A., 1952. Use of dehydroisoandrosterone in psychiatric treatment; a preliminary survey. *British medical journal* 2, 64-66.
- Strohle, A., Romeo, E., di Michele, F., Pasini, A., Hermann, B., Gajewsky, G., Holsboer, F., Rupprecht, R., 2003. Induced panic attacks shift gamma-aminobutyric acid type A receptor modulatory neuroactive steroid composition in patients with panic disorder: preliminary results. *Archives of general psychiatry* 60, 161-168.

- Stromberg, J., Haage, D., Taube, M., Backstrom, T., Lundgren, P., 2006. Neurosteroid modulation of allopregnanolone and GABA effect on the GABA-A receptor. *Neuroscience* 143, 73-81.
- Strous, R.D., Maayan, R., Lapidus, R., Goredetsky, L., Zeldich, E., Kotler, M., Weizman, A., 2004. Increased circulatory dehydroepiandrosterone and dehydroepiandrosterone-sulphate in first-episode schizophrenia: relationship to gender, aggression and symptomatology. *Schizophrenia research* 71, 427-434.
- Strous, R.D., Stryker, R., Maayan, R., Gal, G., Viglin, D., Katz, E., Eisner, D., Weizman, A., 2007. Analysis of clinical symptomatology, extrapyramidal symptoms and neurocognitive dysfunction following dehydroepiandrosterone (DHEA) administration in olanzapine treated schizophrenia patients: a randomized, double-blind placebo controlled trial. *Psychoneuroendocrinology* 32, 96-105.
- Su, Q.R., Su, L.Y., Su, H.R., Chen, Q., Ren, G.Y., Yin, Y., Shen, S.Q., Yu, A.Y., Xia, G.Y., 2007. Polymorphisms of androgen receptor gene in childhood and adolescent males with first-onset major depressive disorder and association with related symptomatology. *The International journal of neuroscience* 117, 903-917.
- Su, T.P., London, E.D., Jaffe, J.H., 1988. Steroid binding at sigma receptors suggests a link between endocrine, nervous, and immune systems. *Science* (New York, N.Y.) 240, 219-221.
- Sullivan, S.D., Moenter, S.M., 2003. Neurosteroids alter gamma-aminobutyric acid postsynaptic currents in gonadotropin-releasing hormone neurons: a possible mechanism for direct steroidal control. *Endocrinology* 144, 4366-4375.
- Suzuki, T., Sasano, H., Andersson, S., Mason, J.I., 2000. 3beta-hydroxysteroid dehydrogenase/delta5-->4-isomerase activity associated with the human 17beta-hydroxysteroid dehydrogenase type 2 isoform. *The Journal of clinical endocrinology and metabolism* 85, 3669-3672.
- Swann, A.C., Bowden, C.L., Morris, D., Calabrese, J.R., Petty, F., Small, J., Dilsaver, S.C., Davis, J.M., 1997. Depression during mania. Treatment response to lithium or divalproex. *Archives of general psychiatry* 54, 37-42.
- Swann, A.C., Secunda, S.K., Katz, M.M., Croughan, J., Bowden, C.L., Koslow, S.H., Berman, N., Stokes, P.E., 1993. Specificity of mixed affective states: clinical comparison of dysphoric mania and agitated depression. *Journal of affective disorders* 28, 81-89.
- Tabori, N.E., Stewart, L.S., Znamensky, V., Romeo, R.D., Alves, S.E., McEwen, B.S., Milner, T.A., 2005. Ultrastructural evidence that androgen receptors are located at extranuclear sites in the rat hippocampal formation. *Neuroscience* 130, 151-163.
- Tagliaferro, A.R., Davis, J.R., Truchon, S., Van Hamont, N., 1986. Effects of dehydroepiandrosterone acetate on metabolism, body weight and composition of male and female rats. *The Journal of nutrition* 116, 1977-1983.
- Taherianfard, M., Shariaty, M., 2004. Evaluation of serum steroid hormones in schizophrenic patients. *Indian journal of medical sciences* 58, 3-9.
- Takebayashi, M., Kagaya, A., Uchitomi, Y., Kugaya, A., Muraoka, M., Yokota, N., Horiguchi, J., Yamawaki, S., 1998. Plasma dehydroepiandrosterone sulfate in unipolar major depression. Short communication. *J Neural Transm* 105, 537-542.
- Takizawa, I., Nishiyama, T., Hara, N., Hoshii, T., Ishizaki, F., Miyashiro, Y., Takahashi, K., 2010. Trilostane, an inhibitor of 3beta-hydroxysteroid dehydrogenase, has an agonistic activity on androgen receptor in human prostate cancer cells. *Cancer letters* 297, 226-230.
- Tan, J., Sharief, Y., Hamil, K.G., Gregory, C.W., Zang, D.Y., Sar, M., Gumerlock, P.H., deVere White, R.W., Pretlow, T.G., Harris, S.E., Wilson, E.M., Mohler, J.L., French, F.S., 1997. Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. *Molecular endocrinology* (Baltimore, Md) 11, 450-459.
- Tedla, Y., Shibre, T., Ali, O., Tadele, G., Woldeamanuel, Y., Asrat, D., Aseffa, A., Mihret, W., Abebe, M., Alem, A., Medhin, G., Habte, A., 2011. Serum antibodies to *Toxoplasma gondii* and *Herpesviridae* family viruses in individuals with schizophrenia and bipolar disorder: a case-control study. *Ethiopian medical journal* 49, 211-220.
- Ter-Minassian, M., Asomaning, K., Zhao, Y., Chen, F., Su, L., Carmella, S.G., Lin, X., Hecht, S.S., Christiani, D.C., 2011. Genetic variability in the metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). *International journal of cancer*.
- Thilers, P.P., Macdonald, S.W., Herlitz, A., 2006. The association between endogenous free testosterone and cognitive performance: a population-based study in 35 to 90 year-old men and women. *Psychoneuroendocrinology* 31, 565-576.
- Tohen, M., Waternaux, C.M., Tsuang, M.T., 1990. Outcome in Mania. A 4-year prospective follow-up of 75 patients utilizing survival analysis. *Archives of general psychiatry* 47, 1106-1111.
- Torres, J.M., Ortega, E., 2003. Precise quantitation of 5alpha-reductase type 1 mRNA by RT-PCR in rat liver and its positive regulation by testosterone and dihydrotestosterone. *Biochemical and biophysical research communications* 308, 469-473.

- Tourney, G., Erb, J.L., 1979. Temporal variations in androgens and stress hormones in control and schizophrenic subjects. *Biological psychiatry* 14, 395-404.
- Tourney, G., Hatfield, L., 1972. Plasma androgens in male schizophrenics. *Archives of general psychiatry* 27, 753-755.
- Toyohara, J., Sakata, M., Ishiwata, K., 2009. Imaging of sigma1 receptors in the human brain using PET and [¹¹C]SA4503. *Central nervous system agents in medicinal chemistry* 9, 190-196.
- Tria, A., Hiort, O., Sinnecker, G.H., 2004. Steroid 5alpha-reductase 1 polymorphisms and testosterone/dihydrotestosterone ratio in male patients with hypospadias. *Hormone research* 61, 180-183.
- Tripodanakis, J., Markianos, M., Rouvali, O., Istikoglou, C., 2007. Gonadal axis hormones in psychiatric male patients after a suicide attempt. *European archives of psychiatry and clinical neuroscience* 257, 135-139.
- Trotter, A., Bokelmann, B., Sorgo, W., Bechinger-Kornhuber, D., Heinemann, H., Schmucker, G., Oesterle, M., Kohntop, B., Brisch, K.H., Pohlandt, F., 2001. Follow-up examination at the age of 15 months of extremely preterm infants after postnatal estradiol and progesterone replacement. *The Journal of clinical endocrinology and metabolism* 86, 601-603.
- Uher, R., Caspi, A., Houts, R., Sugden, K., Williams, B., Poulton, R., Moffitt, T.E., 2011. Serotonin transporter gene moderates childhood maltreatment's effects on persistent but not single-episode depression: replications and implications for resolving inconsistent results. *Journal of affective disorders* 135, 56-65.
- Uzunova, V., Sheline, Y., Davis, J.M., Rasmusson, A., Uzunov, D.P., Costa, E., Guidotti, A., 1998. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. *Proceedings of the National Academy of Sciences of the United States of America* 95, 3239-3244.
- Vacheron-Trystram, M.N., Cheref, S., Gauillard, J., Plas, J., 2002. [A case report of mania precipitated by use of DHEA]. *L'Encephale* 28, 563-566.
- Wagner, C.K., 2008. Progesterone receptors and neural development: a gap between bench and bedside? *Endocrinology* 149, 2743-2749.
- Walder, D.J., Andersson, T.L., McMillan, A.L., Breedlove, S.M., Walker, E.F., 2006. Sex differences in digit ratio (2D:4D) are disrupted in adolescents with schizotypal personality disorder: altered prenatal gonadal hormone levels as a risk factor. *Schizophrenia research* 86, 118-122.
- Valenti, G., Ferrucci, L., Lauretani, F., Ceresini, G., Bandinelli, S., Luci, M., Ceda, G., Maggio, M., Schwartz, R.S., 2009. Dehydroepiandrosterone sulfate and cognitive function in the elderly: The INCHIANTI Study. *Journal of endocrinological investigation* 32, 766-772.
- Valimaki, M., Pelkonen, R., Harkonen, M., Tuomala, P., Koistinen, P., Roine, R., Ylikahri, R., 1990. Pituitary-gonadal hormones and adrenal androgens in non-cirrhotic female alcoholics after cessation of alcohol intake. *European journal of clinical investigation* 20, 177-181.
- Valimaki, M.J., Laitinen, K., Tiitinen, A., Steman, U.H., Ylostalo, P., 1995. Gonadal function and morphology in non-cirrhotic female alcoholics: a controlled study with hormone measurements and ultrasonography. *Acta obstetrica et gynecologica Scandinavica* 74, 462-466.
- Walsh, S., Zmuda, J.M., Cauley, J.A., Shea, P.R., Metter, E.J., Hurley, B.F., Ferrell, R.E., Roth, S.M., 2005. Androgen receptor CAG repeat polymorphism is associated with fat-free mass in men. *J Appl Physiol* 98, 132-137.
- van Goozen, S.H., Matthys, W., Cohen-Kettenis, P.T., Thijssen, J.H., van Engeland, H., 1998. Adrenal androgens and aggression in conduct disorder prepubertal boys and normal controls. *Biological psychiatry* 43, 156-158.
- Van Ness, B., Ramos, C., Haznadar, M., Hoering, A., Haessler, J., Crowley, J., Jacobus, S., Oken, M., Rajkumar, V., Greipp, P., Barlogie, B., Durie, B., Katz, M., Atluri, G., Fang, G., Gupta, R., Steinbach, M., Kumar, V., Mushlin, R., Johnson, D., Morgan, G., 2008. Genomic variation in myeloma: design, content, and initial application of the Bank On A Cure SNP Panel to detect associations with progression-free survival. *BMC medicine* 6, 26.
- van Niekerk, J.K., Huppert, F.A., Herbert, J., 2001. Salivary cortisol and DHEA: association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. *Psychoneuroendocrinology* 26, 591-612.
- Van Pottelbergh, I., Lumbroso, S., Goemaere, S., Sultan, C., Kaufman, J.M., 2001. Lack of influence of the androgen receptor gene CAG-repeat polymorphism on sex steroid status and bone metabolism in elderly men. *Clinical endocrinology* 55, 659-666.
- van Rijn, S., Aleman, A., de Sonnevile, L., Sprong, M., Ziermans, T., Schothorst, P., van Engeland, H., Swaab, H., 2011. Neuroendocrine markers of high risk for psychosis: salivary testosterone in adolescent boys with prodromal symptoms. *Psychological medicine*, 1-8.
- van Wingen, G., van Broekhoven, F., Verkes, R.J., Petersson, K.M., Backstrom, T., Buitelaar, J., Fernandez, G., 2007. How progesterone impairs memory for biologically salient stimuli in healthy young women. *J Neurosci* 27, 11416-11423.

- van Wingen, G.A., van Broekhoven, F., Verkes, R.J., Petersson, K.M., Backstrom, T., Buitelaar, J.K., Fernandez, G., 2008. Progesterone selectively increases amygdala reactivity in women. *Molecular psychiatry* 13, 325-333.
- van Wingen, G.A., Zylicz, S.A., Pieters, S., Mattern, C., Verkes, R.J., Buitelaar, J.K., Fernandez, G., 2009. Testosterone increases amygdala reactivity in middle-aged women to a young adulthood level. *Neuropsychopharmacology* 34, 539-547.
- Wang, C., Marx, C.E., Morrow, A.L., Wilson, W.A., Moore, S.D., 2007. Neurosteroid modulation of GABAergic neurotransmission in the central amygdala: a role for NMDA receptors. *Neuroscience letters* 415, 118-123.
- Wang, C., Tao, W., Chen, Q., Hu, H., Wen, X.Y., Han, R., 2010. SRD5A2 V89L polymorphism and prostate cancer risk: a meta-analysis. *The Prostate* 70, 170-178.
- Wang, D.Y., Bulbrook, R.D., Sneddon, A., Hamilton, T., 1967. The metabolic clearance rates of dehydroepiandrosterone, testosterone and their sulphate esters in man, rat and rabbit. *The Journal of endocrinology* 38, 307-318.
- Wang, J.M., Johnston, P.B., Ball, B.G., Brinton, R.D., 2005. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. *J Neurosci* 25, 4706-4718.
- Wechsler, D., 1999. Wechsler Adult Intelligence Scale - third edition. Pearson Assessments.
- Weill-Engerer, S., David, J.P., Szadovitch, V., Liere, P., Eychenne, B., Pianos, A., Schumacher, M., Delacourte, A., Baulieu, E.E., Akwa, Y., 2002. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *The Journal of clinical endocrinology and metabolism* 87, 5138-5143.
- Weill-Engerer, S., David, J.P., Szadovitch, V., Liere, P., Schumacher, M., Delacourte, A., Baulieu, E.E., Akwa, Y., 2003. In vitro metabolism of dehydroepiandrosterone (DHEA) to 7alpha-hydroxy-DHEA and Delta5-androstene-3beta,17beta-diol in specific regions of the aging brain from Alzheimer's and non-demented patients. *Brain Res* 969, 117-125.
- Weiner, M., Warren, L., Fiedorowicz, J.G., 2011. Cardiovascular morbidity and mortality in bipolar disorder. *Ann Clin Psychiatry* 23, 40-47.
- Vermeersch, H., T'Sjoen, G., Kaufman, J.M., Vincke, J., Van Houtte, M., 2010. Testosterone, androgen receptor gene CAG repeat length, mood and behaviour in adolescent males. *European journal of endocrinology / European Federation of Endocrine Societies* 163, 319-328.
- Vermeulen, A., Verdonck, L., Kaufman, J.M., 1999. A critical evaluation of simple methods for the estimation of free testosterone in serum. *The Journal of clinical endocrinology and metabolism* 84, 3666-3672.
- Westberg, L., Baghaei, F., Rosmond, R., Hellstrand, M., Landen, M., Jansson, M., Holm, G., Bjorntorp, P., Eriksson, E., 2001. Polymorphisms of the androgen receptor gene and the estrogen receptor beta gene are associated with androgen levels in women. *The Journal of clinical endocrinology and metabolism* 86, 2562-2568.
- Weygandt, W., 1899. *Über die Mischzustände des Manisch-Depressiven Irreseins*. In: Lehman, J.F. (Ed.), Munich.
- Whalley, H.C., Baig, B.J., Hall, J., Job, D.E., McIntosh, A.M., Cunningham-Owens, D.G., Johnstone, E.C., Lawrie, S.M., 2010. Effects of the BDNF val66met polymorphism on prefrontal brain function in a population at high genetic risk of schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 153B, 1474-1482.
- WHO, 1977. *The International Classification of Disease - 9th edition*. World Health Organisation, Geneva.
- WHO, 1993. *The ICD-10 Classification of Mental and Behavioural Disorders - Diagnostic Criteria for Research*. World Health Organisation, Geneva.
- Viau, V., Soriano, L., Dallman, M.F., 2001. Androgens alter corticotropin releasing hormone and arginine vasopressin mRNA within forebrain sites known to regulate activity in the hypothalamic-pituitary-adrenal axis. *Journal of neuroendocrinology* 13, 442-452.
- Vieta, E., Morralla, C., 2010. Prevalence of mixed mania using 3 definitions. *Journal of affective disorders* 125, 61-73.
- Wilkins, J.N., Majewska, M.D., Van Gorp, W., Li, S.H., Hinken, C., Plotkin, D., Setoda, D., 2005. DHEAS and POMS measures identify cocaine dependence treatment outcome. *Psychoneuroendocrinology* 30, 18-28.
- Wilson, J.D., 1998. *Endocrinology*. In: Fauci, A.S., Braunwald, E., Isselbacher, K.J., Wilson, J.D., Martin, J.B., Kasper, D.L., Hauser, S.L., Longo, D.L. (Eds.), *Harrisons Principles of Internal Medicine*. McGraw-Hill, pp. 1974-1975.
- Wilson, S.T., Stanley, B., Oquendo, M.A., Goldberg, P., Zalsman, G., Mann, J.J., 2007. Comparing impulsiveness, hostility, and depression in borderline personality disorder and bipolar II disorder. *The Journal of clinical psychiatry* 68, 1533-1539.
- Vogl, G., Zaudig, M., 1985. Investigation of operationalized diagnostic criteria in the diagnosis of schizoaffective and cycloid psychoses. *Comprehensive psychiatry* 26, 1-10.

- Wolf, O.T., Kirschbaum, C., 1999. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. *Brain Res Brain Res Rev* 30, 264-288.
- Wolf, O.T., Neumann, O., Hellhammer, D.H., Geiben, A.C., Strasburger, C.J., Dressendorfer, R.A., Pirke, K.M., Kirschbaum, C., 1997. Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. *The Journal of clinical endocrinology and metabolism* 82, 2363-2367.
- Volz, H.P., Stoll, K.D., 2004. Clinical trials with sigma ligands. *Pharmacopsychiatry* 37 Suppl 3, S214-220.
- Xu, M.Q., St Clair, D., Feng, G.Y., Lin, Z.G., He, G., Li, X., He, L., 2008. BDNF gene is a genetic risk factor for schizophrenia and is related to the chlorpromazine-induced extrapyramidal syndrome in the Chinese population. *Pharmacogenetics and genomics* 18, 449-457.
- Xu, Y.T., Kaushal, N., Shaikh, J., Wilson, L.L., Mesangeau, C., McCurdy, C.R., Matsumoto, R.R., 2010. A novel substituted piperazine, CM156, attenuates the stimulant and toxic effects of cocaine in mice. *The Journal of pharmacology and experimental therapeutics* 333, 491-500.
- Yamada, S., Akishita, M., Fukai, S., Ogawa, S., Yamaguchi, K., Matsuyama, J., Kozaki, K., Toba, K., Ouchi, Y., 2010. Effects of dehydroepiandrosterone supplementation on cognitive function and activities of daily living in older women with mild to moderate cognitive impairment. *Geriatrics & gerontology international* 10, 280-287.
- Yang, L.Y., Verhovshek, T., Sengelaub, D.R., 2004. Brain-derived neurotrophic factor and androgen interact in the maintenance of dendritic morphology in a sexually dimorphic rat spinal nucleus. *Endocrinology* 145, 161-168.
- Yang, S.C., Shieh, K.R., 2007. Gonadal hormones-mediated effects on the stimulation of dopamine turnover in mesolimbic and nigrostriatal systems by cocaine- and amphetamine-regulated transcript (CART) peptide in male rats. *Neuropharmacology* 53, 801-809.
- Yatham, L.N., Kennedy, S.H., O'Donovan, C., Parikh, S., MacQueen, G., McIntyre, R., Sharma, V., Silverstone, P., Alda, M., Baruch, P., Beaulieu, S., Daigneault, A., Milev, R., Young, L.T., Ravindran, A., Schaffer, A., Connolly, M., Gorman, C.P., 2005. Canadian Network for Mood and Anxiety Treatments (CANMAT) guidelines for the management of patients with bipolar disorder: consensus and controversies. *Bipolar disorders* 7 Suppl 3, 5-69.
- Yeap, B.B., 2010. Androgens and cardiovascular disease. *Current opinion in endocrinology, diabetes, and obesity* 17, 269-276.
- Yehuda, R., Brand, S.R., Golier, J.A., Yang, R.K., 2006. Clinical correlates of DHEA associated with post-traumatic stress disorder. *Acta psychiatrica Scandinavica* 114, 187-193.
- Yonker, J.E., Eriksson, E., Nilsson, L.G., Herlitz, A., 2006. Negative association of testosterone on spatial visualization in 35 to 80 year old men. *Cortex; a journal devoted to the study of the nervous system and behavior* 42, 376-386.
- Young, A.H., Gallagher, P., Porter, R.J., 2002. Elevation of the cortisol-dehydroepiandrosterone ratio in drug-free depressed patients. *The American journal of psychiatry* 159, 1237-1239.
- Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry* 133, 429-435.
- Yu, L., Romero, D.G., Gomez-Sanchez, C.E., Gomez-Sanchez, E.P., 2002. Steroidogenic enzyme gene expression in the human brain. *Molecular and cellular endocrinology* 190, 9-17.
- Zanelli, J., Reichenberg, A., Morgan, K., Fearon, P., Kravariti, E., Dazzan, P., Morgan, C., Zanelli, C., Demjaha, A., Jones, P.B., Doody, G.A., Kapur, S., Murray, R.M., 2010. Specific and generalized neuropsychological deficits: a comparison of patients with various first-episode psychosis presentations. *The American journal of psychiatry* 167, 78-85.
- Zhang, L., Li, B., Ma, W., Barker, J.L., Chang, Y.H., Zhao, W., Rubinow, D.R., 2002. Dehydroepiandrosterone (DHEA) and its sulfated derivative (DHEAS) regulate apoptosis during neurogenesis by triggering the Akt signaling pathway in opposing ways. *Brain research* 98, 58-66.
- Zhao, B., Hu, G.X., Chu, Y., Jin, X., Gong, S., Akingbemi, B.T., Zhang, Z., Zirkin, B.R., Ge, R.S., 2010. Inhibition of human and rat 3beta-hydroxysteroid dehydrogenase and 17beta-hydroxysteroid dehydrogenase 3 activities by perfluoroalkylated substances. *Chemico-biological interactions* 188, 38-43.
- Zhu, W., 2008. CYP2D6: a key enzyme in morphine synthesis in animals. *Med Sci Monit* 14, SC15-18.
- Zhuo, F.L., Xu, W., Wang, L., Wu, Y., Xu, Z.L., Zhao, J.Y., 2011. Androgen receptor gene polymorphisms and risk for androgenetic alopecia: a meta-analysis. *Clinical and experimental dermatology*.
- Zitzmann, M., 2009. Testosterone deficiency, insulin resistance and the metabolic syndrome. *Nature reviews* 5, 673-681.
- Zitzmann, M., Weckesser, M., Schober, O., Nieschlag, E., 2001. Changes in cerebral glucose metabolism and visuospatial capability in hypogonadal males under testosterone substitution therapy. *Exp Clin Endocrinol Diabetes* 109, 302-304.

Zofkova, I., Hill, M., Zajickova, K., 2002a. Dehydroepiandrosterone status in postmenopausal women is determined by the gene for the vitamin D receptor. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* 34, 127-131.

Zofkova, I., Zajickova, K., Hill, M., Horinek, A., 2002b. Apolipoprotein E gene determines serum testosterone and dehydroepiandrosterone levels in postmenopausal women. *European journal of endocrinology / European Federation of Endocrine Societies* 147, 503-506.

Zwain, I.H., Yen, S.S., 1999a. Dehydroepiandrosterone: biosynthesis and metabolism in the brain. *Endocrinology* 140, 880-887.

Zwain, I.H., Yen, S.S., 1999b. Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology* 140, 3843-3852.