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Neuropeptide S and mental health: A functional receptor gene variant and environment shaping traits and contributing to psychiatric disorders

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Department of Psychology, University of Tartu, Estonia

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Memories warm you up from the inside.
But they also tear you apart.

Haruki Murakami
Kafka on the Shore

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, further referred to by respective Roman numerals:

I. **Laas K**, Reif A, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) A functional NPSR1 gene variant and environment shape personality and impulsive action: A longitudinal study. *Journal of Psychopharmacology* 28:227–236.

II. **Laas K**, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) Interaction of the neuropeptide S receptor gene $Asn^{107}Ile$ variant and environment: contribution to affective and anxiety disorders, and suicidal behaviour. *International Journal of Neuropsychopharmacology* 17:541–552.

III. **Laas K**, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) Neuropeptide S receptor gene variant and environment: Contribution to alcohol use disorders and alcohol consumption. *Addiction Biology (in press)*. doi:10.1111/adb.12149

IV. **Laas K**, Eensoo D, Paaver M, Reif A, Lesch KP, Harro J Further evidence for the association of the *NPSR1* gene polymorphism ($Asn^{107}Ile$) with impulsivity and hyperactivity. (Manuscript submitted for publication.)

V. **Laas K**, Reif A, Mäestu J, Domschke K, Lesch KP, Veidebaum T, Harro, J Neuropeptide S receptor gene (*NPSR1*) A/T polymorphism and sleep: longitudinal analysis in a population-representative sample. (In manuscript.)

Contribution of the author

- For all the papers, the author of the dissertation formulated research hypotheses, conducted the data analysis, wrote the first draft of the manuscript and was responsible for the final form.
- The author of the dissertation also participated in the ECPBHS data collection and relevant methods development throughout the years of her PhD studies.

ABBREVIATIONS

ADHD	attention deficit hyperactivity disorder
AMIS	Adaptive and Maladaptive Impulsivity Scale
AUD	alcohol use disorder
BIS	Barratt Impulsiveness Scale
FR	family relations
CNS	central nervous system
CRF	corticotropin releasing factor
DNA	deoxyribonucleic acid
ECPBHS	Estonian Children Personality, Behaviour and Health Study
EPSTB	Estonian Psychobiological Study of Traffic Behaviour
G × E	gene-by-environment interaction
HPA axis	hypothalamic-pituitary-adrenal axis
mRNA	messenger ribonucleic acid
NPS	neuropeptide S
NPSR	neuropeptide S receptor
NPSR1	neuropeptide S receptor gene
PCR	polymerase chain reaction
SLE	stressful life events
SNAP-IV	Swanson, Nolan and Pelham Questionnaire version IV

1. INTRODUCTION AND REVIEW OF LITERATURE

The heredity of behavioural traits in humans was obvious already to Plato (427–347 B.C.) who in Book V of the *Republic* (Plato, trans. 1901) described an ideal state that is ruled by the elite class. He suggested that matching the best with the best, and rearing their offspring with attention would lead to the expected, beneficial results as projected from the parental characteristics. However, in addition to his notice of the need of *rearing with attention*, he also understood that ideal provenance is not infallibly predictive and suggested universal education to all citizens, so that the inferior offspring of elite class could be demoted, and the offspring of the lower class who show merit could be promoted. Considering the extreme standpoints in the history of nature-nurture controversy, Plato represented a rather balanced view of the roles of both nature and nurture. However, the specific nature of both heredity and environment, and interplay between them, is still only partly understood. Desire for knowledge and need for new treatments drives research towards understanding how the environment works with the genes to shape behaviour and development of disorders.

1.1. Psychiatric disorders, genetic vulnerability and environment

1.1.1. Environment and stress

The theory of stress and general adaptive syndrome was first proposed by Hans Selye (1936) as a theory of universal bodily responses of the organism under adverse conditions. So stress is a global response of the body to any demand or stressor, external or internal. This groundbreaking theory has since then been transformed to many more specific versions, including those focusing on individual coping styles where stress responses are considered triggered by experiences only if the individual perceives them adverse (Goldstein & Kopin 2007).

Stress responses may follow a time-dependent pattern in which short-term stressors elicit adaptive changes by the organism whereas long-term or chronic stressors evoke maladaptive changes, observable as detrimental changes in various physiological systems, including the CNS (Chrousos 2009; Luine 2007; Nugent et al. 2011). Stress reactions are orchestrated by the activity in the hypothalamus-pituitary-adrenal (HPA) axis, a neuroendocrine system involved in neural, hormonal, and behavioural responses to stressors. Abnormalities in HPA function as a result of stress overload involve changes in monoaminergic, glucocorticoid, and neuropeptide systems, largely triggered by corticotropin-releasing factor (CRF) release (Chrousos 2009; Gillespie et al. 2009). Long-term persistent alterations in these systems may lead to psychiatric disorders,

including mood and anxiety disorders and substance use disorders, sometimes referred to as disorders of the stress system (Chrousos 2009).

Stress responses have repeatedly been documented as different in males and females (reviewed by Luine 2007). Effects of the same chronic stressor on cognition and anxiety have been shown to differ by sex (Bowman et al. 2009; Kumsta et al. 2007). This may be because hormonal and other physiological systems differ in males and females, but may also suggest that males and females perceive the same stressor differently. Indeed, stress- and sex-dependent neurochemical changes have been documented in both humans and animals (Bangasser et al. 2010; Marin et al. 2011). The most natural way of explaining sex differences is derived from distinct endocrine environments in males and females: counteractions take place between the HPA axis and the hypothalamo–pituitary–gonadal (HPG) axis, from which sex hormones are the end products. While cortisol has inhibitory effects at the HPG-axis, hypothalamic regulation of the HPA axis is suppressed by testosterone (Terburg, Morgan & van Honk 2009) explaining why the sexes differ in stress-induced HPA activity (Kudielka & Kirschbaum 2005; Marin et al. 2011). These differences are also thought to explain sex differences in the prevalence of affective, anxiety and substance use disorders (Bangasser et al. 2010; Luine 2007; Witt 2007).

While considering life stress and environment in relation to psychiatric disorders, controversies remain concerning which aspects of life stress are important and how to measure stressors (reviewed by Monroe 2008). Research has documented a wide range of adverse experiences and chronic stresses like war, natural disaster, adverse family environment or severe maltreatment. Stress reactions begin already in prenatal environment and continue throughout life into late years. Much of it apparently remains well compensated for. There seems to be an agreement of opinion that the most detrimental stress originates from early developmental stages up to adolescence (Chrousos 2009; Luine 2007; Nugent et al. 2011). So exposures to trauma, maltreatment and neglect in childhood have been identified as major risk factors for psychopathology in adulthood, especially for mood and anxiety disorders (Gillespie et al. 2009). However, individuals have a wide variety of responses to stress and not all the subjects with history of stressful life events develop a psychiatric disorder. While some individuals are sensitive to adverse experiences and develop a psychopathology, others seem to be resilient by staying symptom free even after experiencing significant adversities. Rutter (2006) has defined resilience as "implying a relative resistance to environmental risk experiences, or the overcoming of stress or adversity". Sources of resilience come from psychological and social resources as well as from genetic factors. According to twin studies, resilience is highly heritable (Boardman, Blalock & Button 2008; Rutter 2006). So it is suggested that high levels of early life trauma lead to disease through the developmental interaction of genetic variants with neural circuits that regulate emotion, together mediating risk and resilience in adults (Gillespie et al. 2009).

1.1.2. Personality and psychiatric risk

Personality and psychopathology have been relatively distinct research objects, one being rather dealt with in psychology and the other as the domain of psychiatry. Researchers have largely agreed about the existence of basic temperaments, and while normal ranges of temperament manifest as personality, extremes of the temperament confer the risk of psychopathology. Proposals for the construction of a structurally valid model of psychopathology that draws on the structure of personality for both Axis I and Axis II disorders have been made (e.g., Kruger & Eaton 2010).

Mood and anxiety disorders are the most common psychiatric disorders with 12 month prevalence in European countries of 14% and 7.8%, respectively (Wittchen et al. 2011). Mood and anxiety disorders have significant comorbidity with each other and also with alcohol use disorders: among subjects with lifetime affective disorder, 41.4% had lifetime anxiety disorder and 40.3% had lifetime alcohol use disorder (Hasin et al. 2005). Affective disorders and comorbidity with alcohol use and anxiety disorders are associated with high suicidality (Wasserman 2001).

Males and females have different prevalence of affective, anxiety and substance use disorders. While mood and anxiety disorders are at least twice as common in females compared to males, substance use is at least three times more common in males (e.g., Wittchen et al. 2011).

Psychiatric diagnoses overlap significantly with extremes of dimensional traits, such as Big Five personality dimensions, impulsivity, anxiety, depressiveness and self-esteem. This indicates shared, common etiology or influence on one another. For example, higher neuroticism and lower extraversion predict major depression, and the association arises largely because neuroticism shares genetic risk with depression (Kendler et al. 2006). On the other hand, anxiety is not specific for anxiety disorders only, e.g., high anxiety often accompanies and precedes an affective disorder even when no anxiety disorder was diagnosed (Moffitt et al. 2007; Wassermann 2001). Anxiety and neuroticism are associated also with substance abuse and dependence and all these constructs are associated with impulsivity and extraversion (Acton 2003; Chartier, Hesselbrock & Hesselbrock 2010; Ersche et al. 2010; Iacono, Malone & McGue 2003). Subjects with affective/anxiety disorders and suicidal behaviour usually exhibit low self-esteem (Mann et al. 2003; Wassermann 2001; Wild, Flisher & Lombard 2004). Self-esteem, the affective or evaluative appraisal of one's self, is linked with adaptive personality functioning and has substantial genetic origin (Neiss, Sedikies & Stevenson 2002).

The high heritability (Burmeister, McInnis & Zöllner 2008) and comorbidity of mood, anxiety and substance use disorders suggest shared genetic and environmental factors (Domschke & Reif 2012; Heinz et al. 2001; Kendler et al. 2008; Kertes et al. 2011; Levine et al. 2001;) which are more closely discussed below.

1.1.3. Psychiatric risk alleles and gene-environment interactions

Much of the evidence of genetic background of mental health, and more specifically on affective and anxiety disorders, is derived from studies on monoaminergic systems. This is because of the relative success of the candidate gene approach that has placed focus on detection of functional variants of genes known to regulate neuronal activity that is affected by known psychoactive drugs. The effects of common genetic variants are however difficult to detect as these only have small effects on phenotype.

In addition, alleles mostly exert their influence under specific environmental conditions, as shown in the pioneering studies of Caspi and colleagues on monoaminergic genes, behaviour and depression (Caspi et al. 2002; Caspi et al. 2003). Since then, the number of studies considering G × E effects is ever increasing. As a complex disorder, depression is caused by both biological and environmental factors, and has been suggested to have disturbances in monoamine neurotransmission in the CNS (Harro & Oreland 2001; Moret & Briley 2011).

Abnormalities in the HPA axis in response to increased levels of stress are often found to be associated with dysregulations in the serotonergic system, and to be relevant in mood disorders and in suicidal behaviour (Mandelli & Serretti 2013; Pompili et al. 2010). Involvement of the serotonergic dysfunction has also been described at the level of gene variants. As an example, one of the most studied psychiatric risk gene variants is the short allele of the serotonin transporter promotor polymorphism, 5-HTTLPR (Lesch et al. 1996; Lesch 2004) that mediates reactivity to environmental factors: the s-allele carriers show bad mood, less effective adaption, and in some cases depression, in response to environmental adversities (reviewed by Bagdy et al. 2012).

Besides mood disorders, G × E effects on the serotonin and HPA axis contribute to anxiety disorders (reviewed by Nugent et al. 2011). Despite of high heritability (up to 70%, Burmeister et al. 2008), the evidence for G and G × E effects on alcohol dependence and abuse is scarce compared to affective and anxiety disorders (reviewed by Young-Wolff et al. 2010). However, again, monoaminergic genes are the most studied players in alcohol research as well (Young-Wolff et al. 2010).

In addition to deleterious effects that gave them the name, psychiatric risk or vulnerability alleles ought to have also positive effects, otherwise they would go extinct. Indeed, many gene variants that have been associated with higher psychiatric risk have parallel advantages, and these effects are best observable in the background of supportive environment. So Belsky et al. (2009) have suggested a differential susceptibility model where some individuals are not only more sensitive to negative experiences but they react more adaptively also to favourable experiences. Accordingly, the 5-HTTLPR s/s-genotype that has been associated with higher risk for affective dysregulation and suicide risk in the background of stressful life events had the lowest scores of depressiveness and suicidality in the absence of adverse life events (Caspi et al. 2003). Similar

pattern has emerged in other studies: the s-allele carriers were vulnerable to adverse environment, but in the absence of adversities, they had far lower depression scores compared to subjects with no risk allele (e.g., Brummett et al. 2008; Eley et al. 2004).

The progress in understanding complex disorders and traits has not been as fast as expected. In addition to the complexity of their genetic background, the reasons for non-replicable or even contradictory results may lie in differently specified and measured traits, principles of selecting subjects, not accounting for environmental factors or measuring environmental influences differently. Still, a lot can be done for revealing G × E effects by investing into study design – using population-representative samples, conducting longitudinal designs, introducing dimensional phenotypes parallel to the categorical disease phenotypes (Domschke & Reif 2012), and measuring different aspects of environmental stressors.

1.2. Neuropeptide S system

"Neuropeptides are substances with a peptide structure that are synthetized in nervous tissue and used there as messenger molecules. Some neuropeptides fulfill all criteria for a neurotransmitter and may have additional roles as neuromodulators or growth factors" (Encyclopedia of Psychopharmacology). Neuropeptides are involved in the regulation of wide range of physiological functions such as arousal, sleep, reward, metabolism, food intake, reproduction, stress response, learning and memory. Neuropeptide S (NPS) and its G-protein coupled receptor NPSR constitute a relatively novel neuropeptide system that is involved in the regulation of arousal and anxiety, and various other physiological functions.

1.2.1. Biochemistry, chemical neuroanatomy, physiology and pharmacology

Neuropeptide S (NPS) is a 20-amino-acid peptide acting as a neuromodulator by binding to a G-protein coupled receptor, now referred to as NPSR (Xu et al. 2004).

NPS precursor is present in tetrapods, including mammals, birds, reptiles and amphibians, and sequence analysis has revealed that it is evolutionarily highly conserved (Reinscheid 2007). The *N*-terminus is identical in all species suggesting it to be the bioactive part of the NPS. The neuropeptide S receptor, NPSR, is a typical G-protein coupled receptor (Xu et al. 2004) that was first identified as the G-protein coupled receptor 154 (GPR154, Sato et al. 2002), or the vasopressin-like receptor VRR1 (Gupte et al. 2004) as well as the G-protein coupled receptor for asthma susceptibility GPRA (Laitinen et al. 2004). The corresponding encoding gene is located on chromosome 7p14.3. NPSR-like

sequence has been identified in hemichordates and cephalochordates, suggesting an early emergence (Pitti & Manoj 2012). In humans, the closest homologs of NPSR are vasopressin-like receptors (Pitti & Manoj 2012).

Alternative splicing of human NPSR mRNA produces multiple isoforms of NPSR but only few full-length variants produce functional receptors that are transported into the plasma membrane (Laitinen et al. 2004; Reinscheid et al. 2005; Vendelin et al. 2005).

In rat brain, NPS precursor mRNA is highly expressed in the brainstem area between locus coeruleus and the Barrington`s nucleus, while scattered mRNA signals have also been detected in amygdala and hypothalamus (Xu et al. 2004). In contrast, NPSR is expressed in many brain areas including amygdala, thalamus, hypothalamus, hippocampus, preoptic area and orbital cortex (Xu et al. 2004; Xu et al. 2007). The majority of NPS-expressing neurons in the locus coeruleus area and principal sensory trigeminal nucleus are glutamatergic, while NPS neurons in lateral parabrachial nucleus co-express corticotropin releasing factor (Xu et al. 2007). The alongside localisation of the NPS neurons with the noradrenergic neuronal cluster in locus coeruleus suggests that this brainstem area may contain two independent transmitter systems that modulate arousal and vigilance (Xu et al. 2004; see below).

The NPS precursor and receptors are also found outside of CNS: NPS in epithelia of several organs (Vendelin et al. 2005; Xu et al. 2004), and NPSR mRNA in human macrophages and eosinophils (Pulkkinen et al. 2006) where their expression is increased during inflammatory diseases (d´Amato et al. 2010; Camilleri et al. 2010; Laitinen et al. 2004; Vendelin et al. 2005).

NPS activates NPSR at subnanomolar concentrations, consecutively inducing mobilization of intracellular calcium, increasing intracellular cAMP formation, and stimulating phosphorylation of mitogen-activated protein kinase (Reinscheid et al. 2005; Xu et al. 2004).

The NPS system modulates the release and action of other essential neurotransmitters and hormones: NPS enhances glutamatergic neurotransmission in amygdala (Jüngling et al. 2008), stimulates dopaminergic activity in the medial prefrontal cortex and the nucleus accumbens (Mochizuki et al. 2010; Si et al. 2010), and inhibits the release of serotonin and noradrenaline in the frontal cortex (Raiteri et al. 2009). NPS also increases plasma ACTH and corticosterone levels indicating the role of NPS in stimulation of the HPA axis and in stress response (Smith et al. 2006). A role for NPS in the modulation of stress response in rodents was also found by Ebner et al. (2011) where stress led to increased NPS levels in amygdala, and by Chauveau et al. (2012) where NPS injection to amygdala prevented stress-induced changes of aversive behaviours.

So, the localisation of NPS and NPSR mRNA, their co-expression with other neurotransmitters, and impact on the release or action of other bioactive substances all suggest that neurotransmission using NPS modulates various physiological functions like arousal, anxiety, energy homeostasis, stress response, pain, learning and memory. Indeed, the evidence of the functional

involvement of the NPS system in these important functions is accumulating as further described below. However, data from humans remain scarce.

1.2.2. Neuropeptide S receptor gene (*NPSR1*) A/T polymorphism ($Asn^{107}Ile$) in humans

The most extensively studied receptor isoforms expressed in brain are the NPSR1 Asn^{107} and NPSR1 ^{107}Ile. The *NPSR1* gene has an A/T functional single-nucleotide polymorphism (rs324981) that is responsible for an Asn-Ile exchange in the first extracellular loop of the receptor protein at position 107. NPS has up to 10 times higher potency on the ^{107}Ile (T-allele) encoded receptor compared to Asn^{107} (A-allele) in terms of more effective signal transduction and mobilization of intracellular calcium, stimulation of cAMP synthesis and induction of MAPK phosphorylation (Reinscheid et al. 2005). Another NPSR variant, expressed in epithelia of several organs, contains alternatively spliced C-terminus and shows a similar pharmacological profile to NPSR1 Asn^{107} (Reinscheid et al. 2005).

The rs324981 A/T functional polymorphism was soon after initial discovery found to be linked to emotional processing, and anxious, fear- and activity-related traits (Dannlowski et al. 2011; Domschke et al. 2011; Donner et al. 2010; Okamura et al. 2007; Raczka et al. 2010). The precise nature of this association, however, was not easy to characterize and is the subject of this dissertation.

1.2.3. NPS modulates anxiety and arousal

NPS has a rather unusual profile in animal studies: It elicits arousal paralleled by an anxiolytic-like effect (Leonard et al. 2008; Pulga et al. 2012; Reinscheid 2008; Rizzi et al. 2008; Smith et al. 2006; Xu et al. 2004). This has led NPS being called "an activating anxiolytic" (Guerrini et al. 2010; Koob et al. 2004). Specifically, NPS stimulates locomotor activity, increases wakefulness and reduces burying behaviour and other anxiety-related behaviours in both mice and rats (Leonard et al. 2008; Lukas & Neumann 2012; Paneda et al. 2009; Pape et al. 2010; Pulga et al. 2012; Rizzi et al. 2008; Smith et al. 2006; Wegener et al. 2012; Xu et al. 2004; Zhao et al. 2012). In addition, NPS facilitates the extinction of fear-related memory (Lukas & Neumann 2012). The locomotor activity enhancing effect is most likely caused by NPS action on the HPA axis because NPS increased the release of CRF, and the CRF antagonist blocked the locomotor enhancing effect of NPS without affecting its anxiolytic action (Paneda et al. 2009; Smith et al. 2006).

Rodent studies have led to the expectation that the more active NPS-system should be associated with higher arousal also in humans. Indeed, the T-allele of the *NPSR1* gene A/T polymorphism ($Asn^{107}Ile$) that encodes for the more active

receptor isoform has been associated with arousal expressed by higher heart rate (Domschke et al. 2011); stronger reactions to aversive stimuli (Dannlowski et al. 2011; Klauke et al. 2014; Raczka et al. 2010; Tupak et al. 2013); later bedtime in a genome wide study of sleep and circadian rhythm phenotypes (Gottlieb, O'Connor & Wilk 2007); and higher prevalence of panic disorder (Domschke et al. 2011; Donner et al. 2010; Okamura et al. 2007).

Affective and anxiety disorders may develop in consequence of an interplay between biological predisposition and environmental factors like adverse life events and family environment (e.g., Domschke & Reif 2012; Harro 2010). Before studies leading to the present dissertation, there had been only one gene x environment (G × E) interaction study with *NPSR1* probing the Anxiety Sensitivity Index (ASI) (Klauke et al. 2014, published online 2012) which however was limited by the retrospective assessment of life events and the use of a rather specific psychometric scale. A few animal studies with NPS have also directly dealt with stress regulation: forced swim stress induced release of NPS in the amygdala (Ebner et al. 2011), and administration of NPS into the amygdala facilitated extinction of conditioned fear responses (Chauveau et al. 2012; Jüngling et al. 2008). Such evidence would indirectly support the hypothesis that subjects with more effective NPS-ergic neurotransmission, e.g., subjects with the *NPSR1* TT genotype, should be able to deal better with stress, i.e., be more resilient. Nevertheless, as mentioned above, so far the T-allele has rather been associated with psychiatric conditions.

Stress is differently perceived and expressed in males and females, as discussed above, and gender related effects of *NPSR1* have been already reported (Dannlowski et al. 2011; Domschke et al. 2011; Okamura et al. 2007). This is not surprising as the NPS system interacts with the HPA axis (Paneda et al. 2009; Smith et al. 2006). In addition, in the G × E study by Klauke et al. (2014), the *NPSR1* T-allele carriers had higher scores in Anxiety Sensitivity Index if reporting higher number of adverse life events. It follows that Sex × *NPSR1* and Environment × *NPSR1* effects are expected.

1.2.4. NPS system and reward

Central administration of NPS enchances dopaminergic neurotransmission (Mochizuki et al. 2010; Si et al. 2010) that in turn is known to be related to reward-associated behaviour and addiction. In rodents, activity of the NPS system has been associated with addictive behaviours in several studies (reviewed by Cannella et al. 2013) but the direction of the association is equivocal because in some experiments, higher NPS-ergic activity is related to substance seeking (Cannella et al. 2009a; Cao et al. 2011; Paneda et al. 2009), and in others it appears as protective against substance use and dependence (Badia-Elder et al. 2008; Enquist et al. 2012; Ruggeri et al. 2010). In more detail, administration of NPS facilitated reward seeking and induced positive reinforcement (Cao et al. 2011), and reinstated cocaine (Paneda et al. 2009) and

alcohol seeking in rodents (Cannella et al. 2009a). On the other hand, NPS administration decreased alcohol intake in two alcohol-preferring rat lines (Badia-Elder et al. 2008; Cannella et al. 2009b), that are known for their anxious phenotype and therefore hypotesised to consume alcohol for its anxiolytic effects (Cannella et al. 2013). In line with this, Enquist et al. (2012) showed that injection of NPS into the basolateral amygdala promoted anxiolysis after chronic ethanol consumption. So it is possible that, in alcohol preferring rats, NPS decreases ethanol consumption not because it acts on reward mechanisms, but rather because of its anxiolytic-like properties (Cannella et al. 2013).

These seemingly controversial findings, however, are not surprising as low NPS-ergic activity is associated with anxiety, whereas high NPS-ergic activity is linked to arousal, hyperlocomotion and impulsivity (e.g., Rizzi et al. 2008; Xu et al. 2004), and substance use is related to both anxiety and impulsivity (Acton 2003; Chartier et al. 2010; Ersche et al. 2010; Grant et al. 2004; Iacono et al. 2003). However, up to now, there are no studies on NPS or NPSR1 effects on substance use in humans. As NPSR1 is involved in emotional processing and arousal in both humans and animals, and NPS system modulates reward and addiction in animals, the search for evidence of *NPSR1* involvement in substance use and addiction in humans is warranted.

2. AIMS OF THE STUDY

The general aim of this dissertation was to examine the association of a functional *NPSR1* rs324981 A>T polymorphism ($Asn^{107}Ile$) with a variety of behavioural and physiological measures that, based on the literature, could have been hypothesized to be affected by inter-individual differences in NPS-mediated neurotransmission. This was facilitated by access to multidisciplinary databases from large population-derived samples. Particular emphasis was placed on possibility of occurrence of G × E interactions, and on the role gender may play in such interactions.

More specifically, the research questions were:

1. Is the *NPSR1* A/T polymorphism associated with personality traits, and whether any eventual associations depend on age, sex, stressful life events and family environment?
2. Is the *NPSR1* A/T polymorphism associated with impulsivity and ADHD-related traits, and whether any eventual associations depend on age, sex, stressful life events and family environment?
3. Is the *NPSR1* A/T polymorphism associated with depressiveness, anxiety and self-esteem, and whether any eventual associations depend on age, sex, stressful life events and family environment?
4. What is the relationship between the *NPSR1* A/T polymorphism and anxiety and mood disorders, and whether any associations depend on sex and environmental factors?
5. Is the *NPSR1* A/T polymorphism is associated with suicide attempts, and whether the association is modified by sex and environmental factors?
6. Are there links between *NPSR1* A/T polymorphism and alcohol use and alcohol use disorders, and whether any eventual links depend on age, sex, stressful life events and family environment?
7. What are the mediating traits in the possible association of *NPSR1* A/T polymorphism with alcohol use and alcohol use disorders, accounting for sex and environmental factors?
8. Does the *NPSR1* A/T polymorphism affect sleep, and if yes, does this depend on sex and environmental factors?

3. MATERIALS AND METHODS

3.1. Subjects

3.1.1. The Estonian Children Personality Behaviour and Health Study (Papers I, II, III and V)

Data of both birth cohorts of the Estonian sample for the European Youth Heart Study (EYHS, 1998/99), which was subsequently incorporated into the longitudinal Estonian Children Personality Behaviour and Health Study (ECPBHS) were used for most of the analyses in this dissertation. The rationale and procedure of sample formation have been described in detail elsewhere (Harro et al. 2001; Tomson et al. 2011), but it is pertinent to point out that this sample is population-representative and has low attrition rate. The total number of subjects in the first wave in 1998/99 was 1176; 583 in the younger cohort (M_{Age}=9.6±0.5) and 593 in the older cohort (M_{Age}=15.6±0.6). The follow-up studies for the younger cohort took place in 2004 (n=483, M_{Age}=15.3±0.5) and 2007 (n=453, M_{Age}=18.3±0.5); for older cohort, the follow-ups were in 2001 (n=454, including 62 additional subjects, M_{Age}=18.4±0.7) and 2008 (n=540, M_{Age}=24.7±0.7). All subjects were of Caucasian origin. EYHS and ECPBHS have been approved by the Tartu University Ethics Review Committee on Human Research. All participants, and the parents, gave informed consent.

3.1.2. The Estonian Psychobiological Study of Traffic Behaviour (Paper IV)

The database of the Estonian Psychobiological Study of Traffic Behaviour (EPSTB; Eensoo et al. 2010; Paaver et al. 2006; Paaver et al. 2013) that includes two population derived samples was used. One (Car Drivers) was formed of car driving male subjects selected randomly from the driving-licence database of the Estonian Motor Vehicle Registration Centre as a control group to traffic law violators (original N=509; M_{Age}=36.7±11.8; Paaver et al. 2006). These participants filled in impulsivity self-reports during a visit to laboratory in 2001–2003. The other sample (Driving School; original N=1866, M_{Age}=24.0±8.0) was formed during an intervention study in driving schools on students applying for a passenger car driving license (Eensoo et al. 2010; Paaver et al. 2013). In brief, 24 driving schools out of 54 in the two biggest cities in Estonia, Tallinn and Tartu, were considered eligible and agreed to participate. Subjects from the total of 113 study groups in these driving schools filled in questionnaires in a driving school lesson, and some responded to an additional questionnaire sent by e-mail 2–3 years later. We have used data from years 2007 when impulsivity and mild social deviance were measured and 2010 when attention deficit hyperactivity disorder related symptoms were self-reported. Altogether 773 subjects (41%) donated blood samples (males, n=318,

M_{Age}=22.6±7.4; females n=455, M_{Age}=25.1±8.2 during the original sampling). This study was approved by the institutional Ethics Review Committee of University of Tartu, Estonia. All participants gave informed consent.

3.2. Measures

3.2.1. Personality

Personality traits of the five-factor model (**Papers I, II and III**) were measured in ECPBHS by self-reports with the Estonian version of Revised NEO Personality Inventory (NEO-PI-R, Kallasmaa et al. 2000), EPIP-NEO (Mõttus et al. 2006) which is a semantically simplified full-length version of NEO-PI-R, or Estonian Brief Big Five Inventory (EBBFI) which is a shorter and semantically simplified questionnaire (Laidra et al. 2006; Harro et al. 2009). Personality data were collected from the younger cohort at ages 15 (EPIP-NEO) and 18 (EBBFI), and the older cohort at age 15 (EBBFI), 18 and 25 (both NEO-PI-R). As the data have been collected with different instruments all scores were transformed into z-scores for statistical analysis.

3.2.2. Impulsivity and symptoms of attention deficit hyperactivity disorder

Impulsivity was self-reported (**Papers I, III and IV**) by subjects of all samples. We used the Adaptive and Maladaptive Impulsivity Scale (Paaver et al. 2006; Laas et al. 2010) with subscales measuring Fast decision making and Excitement seeking (functional or adaptive impulsivity), and Disinhibition and Thoughtlessness (dysfunctional or maladaptive impulsivity). In ECPBHS, the younger cohort filled AMIS at ages 15 and 18, and the older cohort at ages 18 and 25. Subjects of both Car Drivers and Driving School samples filled AMIS once. We have also used Barratt Impulsivity Scale 11th version (BIS-11, Patton et al. 1995; Estonian version described by Paaver et al. 2007), filled in by the ECPBHS younger cohort at ages 15 and 18, and by the older cohort at age 25; and by the sample of Driving School during initial evaluation.

ADHD symptoms were reported by teachers (Kiive et al. 2010) in ECPBHS at ages 9, 15 and 18 using the Hyperactivity Scale and SNAP-IV (**Papers I and III**). The Hyperactivity Scale consists of three items (Aggressiveness, Motor restlessness, and Concentration difficulties), and Hyperactivity score was calculated by summing the scores of Motor Restlessness and Concentration Difficulties (af Klinteberg & Oreland 1995). Swanson, Nolan & Pelham Questionnaire SNAP-IV consists of two subscales, Inattention and Hyperactivity/ impulsivity (Swanson et al. 2001). Adult ADHD Self-Report Scale (ASRS, Kessler et al. 2005) was administered to the older cohort of ECPBHS at age 25 (**Papers I and III**) and to the Driving School sample about three years after

initial assessment (**Paper IV**). The scales were also dichotomised by upper 5% for the hypothetical presence of ADHD for examining categorical *NPSR1* associations in ECPBHS.

3.2.3. Psychiatric diagnosis

Psychiatric assessment based on DSM-IV was carried out in the older cohort of the ECPBHS at age 25 by experienced clinical psychologists using the Mini-International Neuropsychiatric Interview (MINI 5.0.0; Sheehan et al. 1998; Estonian version: Shlik et al. 1999) at age 25 (**Papers II and III**). Substance use disorders comprised mainly of alcohol use disorders, so three males with *NPSR1* genotype who were abusing or were dependent on illicit drugs were excluded from analysis for the sake of the clarity. We used lifetime prevalence of disorders in this analysis. As of writing this dissertation, psychiatric assessment has not been carried out in the younger cohort.

3.2.4. Suicidality

History of suicide attempts was self-reported by the subjects of the younger cohort of ECPBHS at ages 15 and 18, and by the older cohort at age 18 (**Paper II**). The data of both cohorts at age 18 was combined for analysis. The question asked was: "Have you ever tried to attempt a suicide?" with two possible answers – "Yes" or "No".

3.2.5. Depressiveness

Depressiveness was measured by the self-report version of the Montgomery-Åsberg Depression Rating Scale (MÅDRS; Montgomery & Åsberg 1979) or Beck Depression Inventory (BDI; Beck et al. 1961) (**Paper II)**. Subjects of the younger cohort of the ECPBHS filled BDI at age 15 and MÅDRS at age 18; and subjects of the older cohort filled MÅDRS at ages 18 and 25.

3.2.6. Anxiety

The Spielberger State Trait Anxiety Inventory (STAI, Spielberger 1983) was used to measure anxiety in **Paper II** in the ESPBHS. STAI was administered to subjects of the younger cohort at ages 15 and 18; and to the older cohort at age 25.

3.2.7. Self-esteem

The Rosenberg Self-Esteem Scale (RSES, Rosenberg 1965; Estonian version Pullmann & Allik 2000) was used to measure global self-esteem (**Paper II)**. Self-esteem is defined as a favorable or unfavorable attitude toward the self (Rosenberg 1965). RSES items were self-reported on a 5-point Likert scale ranging from 0 (strongly disagree) to 4 (strongly agree) by subjects of both the older (ages 15 and 18) and the younger cohort (at age 25) of the ECPBHS.

3.2.8. Alcohol use

Alcohol use (**Paper III**) was self-reported during the visit to the laboratory (Merenäkk et al. 2011) in all waves of the ECPBHS. The measures in questionnaires varied by study waves. Alcohol use indices were the age of first drink (the age of having the first ½ standard drink in years); alcohol use (frequency of alcohol use); heavy drinking (frequency of having five or more drinks on a single occasion); being drunk (frequency of drinking to intoxication); getting drunk deliberately (in past, answers yes/no). Questions about alcohol use effects were administered at age 18 to the older cohort only "When I drink alcoholic beverages, I feel relaxed", "When I drink alcoholic beverages I feel happier" (1-very likely, 2-likely, 3-unsure, 4-unlikely, 5-very unlikely). To reduce skewness, alcohol use indices that were not binary were dichotomised except the age of having the first drink.

3.2.9. Family relations

Family relations were self-reported by the subjects of ECPBHS (**Papers I, II, III and V)** with the Tartu Family Relationships Scale (TFRS; Kiive et al. 2010; Paaver et al. 2008). TFRS consists of four subscales named Closeness (15 items, e.g., "Our family is dedicated to each other", "The marriage of my parents is happy"), Support (7 items, e.g., "My family supports me", "Someone in the family helps (has helped) me to feel myself important and special"), Misprize (10 items, e.g., "I can make no decision on my own", "I am depreciated at home"), and emotional and physical Abuse (7 items, e.g., "Were you ever hit by someone in your family or have you experienced physical violence in your family?"). Items were presented on 4 or 5-point Likert scale. Based on similarity, the four subscales can be combined to obtain two higher order scales Warmth (Closeness and Support) and Maltreatment (Neglect/ Misprize and Abuse). A single measure of positive family relationships (FR) was formed by subtracting scores of Maltreatment from scores of Warmth. We have used only higher order scales Warmth, Maltreatment and Family relations. Subjects were divided into low and high groups by median split.

3.2.10. Stressful life events

History of stressful life events (SLE) was self-reported by the subjects of ECPBHS (**Papers I, II, III and V**). The list of adverse life events varied across measurement times and consisted of 10–17 (dependent on the study wave) stressful experiences including parental death and divorce/-separation, unemployed parent, parental alcoholism, poverty, poor living conditions, poor health, accidents and traumas, physical abuse, emotional abuse, severe burden/ serious concerns, suicidal attempts, leaving home for several days without telling anyone, depression of a close relative, committed suicide, or suicide attempt of a close relative (Reif et al. 2011). Subjects were divided into low and high SLE exposure groups by median split.

3.2.11. Mild social deviance

Mild social deviance was measured by the Social Motivation Questionnaire (SMQ, West et al. 1993) in the Driving School sample (**Paper IV**). SMQ is a short instrument consisting of 10 items on a 3-point Likert scale; and it measures mild social deviance in imagined situations, which might harm the interests of others.

3.2.12. Anthropometric measures

Anthropometric measurements were available for ECPBHS and carried out in both cohorts in all waves (subjects with all data including *NPSR1* by study waves: older cohort n=575, 369 and 449, respectively; younger cohort n=563, 469 and 437, respectively). Body mass index (BMI, kg/m^2) was calculated based on height and weight and used as a covariate in **Paper V** because BMI has been found to be associated with sleep duration and quality in adolescents (Shochat et al. 2014).

3.2.13. Sleep duration and bedtime

Sleep-related measures were available for the ECPBHS cohorts (**Paper V**). For the first study wave in 1998/99, sleep duration was calculated from self-reports of bedtime and wake-up time (Ortega et al. 2011). Subjects answered two questions: (1) "What time do you usually get up on a School day?" with four possible answers ranging from <06:30 to >07:30, at 30 min intervals; and (2) "What time do you usually go to bed on a School day?" with four possible answers ranging from <20:00 to >22:00, at 1 h intervals. In next waves, the questions administered to both birth cohorts were: (1) „When do you usually go to bed?" with five possible answers ranging from <21h to >24h, and (2) „How long do you usually sleep?" with five time intervals from <7h to >12h. Sleep

duration was transformed into a two-category variable <9h and >9h for ages 9–18 according to the National Sleep Foundation that defines optimal sleep in children and adolescents as sleeping more than 9 h (National Sleep Foundation 2006). At age 25, five time intervals from <6h to >11h were used, and sleep duration was transformed into a two-category variable <8h and >8h for further analysis. Both bedtime and sleep duration were self-reported.

3.2.14. Sleep-related difficulties

In all the waves, reports of sleep-related difficulties were obtained (**Paper V**): (1) difficulties with falling asleep in the evening; (2) difficulties with getting up in the morning; and (3) tiredness in the morning. Six-point Likert scale was used, from 1 – no difficulties to 6 – difficulties almost every day. We have used parental reports for the younger cohort in all three waves (subjects with genotype data: n=533 at age 9, n=444 at age 15, and n=404 at age 18), and for the older cohort at ages 15 (n=482) and 18 (n=332). At age 25, only self-reports were available (n=471). Composite score of sleep-related problems was formed by summing the scores of all three questions.

3.2.15. *NPSR1* rs324981 A/T polymorphism ($Asn^{107}Ile$) genotyping

The alleles at the *NPSR1* rs324981 ($Asn^{107}Ile$) SNP locus were amplified from DNA isolated from venous blood samples as previously described (Domschke et al. 2011). The genotyping was carried out in the Department of Psychiatry of the University of Wuerzburg, Germany. Briefly, DNA isolated from venous blood samples was amplified by PCR using the primers F: 5`-GAAGGAAAAAAATTAAAAATGAACCTCCCCAGGATTTCAT and R: 5`-TCTACCCAGGAGAAAGCGGGCAGTTTGATGCA. Standard PCR was carried out in a 20-µl volume containing 45–60 ng of genomic DNA, 10 pmol of each primer, 200 µM dNTPs, 0.4 U Taq^{TM} DNA Polymerase (Eppendorf AG, Hamburg, Germany), 50 mM KCl, 1.5 mM $MgCl_2$, and 10 mM Tris-HCl (pH 8.4). After a 5 min denaturation, 35 cycles were carried out each consisting of 30 s at 94 °C, 30 s at 66 °C, and 60 s at 72 °C, followed by a final extension time of 10 min at 72 °C. Amplicons were digested with TasI (Fermentas) (1 U), separated for 2 h on a 15 % polyacrylamide gel and visualized by silver staining. *NPSR1* A/T polymorphism was genotyped in all four samples: in the older (n=575) and in the younger cohort (n=563) of the ECPBHS; and in Car Drivers (n=491) and Driving School (n=773) of the EPSTB. Genotype frequencies were in Hardy-Weinberg equilibrium in all the samples and for both sexes (Table 1).

3.3. Data analysis

The *NPSR1* genotype distribution by categorical variables was compared with Chi-square or Fisher`s exact test; odds ratios (OR) with confidence intervals (CI) were calculated as a measure of effect size (**Papers I–V**). Correlation analysis was used to reveal associations between study variables (**Papers I–V**). Analysis of variance and covariance (AN(C)OVA) was used to test the effect of *NPSR1* and other categorical variables on study variables (**Papers I–V)**. AN(C)OVA results are reported as F-statistic, raw *p*-value, η^2 as a measure of effect size and CI for group means. In figures, whiskers indicate 95% confidence intervals. In **Paper III**, binary logistic stepwise regression with backward elimination of the covariates was used to test if covariates (personality, hyperactivity and environmental factors) explain the relationship between the genotype and outcome variables. A significance level of ≤ 0.20 was used to allow a covariate into the model, and a significance level of ≤ 0.25 for a covariate to stay in the model (we used enter method for the first data block containing genotype effects). The models were built by the age when covariates were reported; e.g., for the older cohort, if alcohol related report was attained at age 15 we used covariates from age 15; and for predicting AUD and alcohol use at age 25, covariates from ages 15, 18 and 25 were used in three separate models per outcome variable. The results from regression models for AUD were reported as regression coefficient β with 95% confidence interval (CI). The results for alcohol use were reported as the direction of the influence of significant covariates and the *p*-value for the genotype effect after accounting for covariates. Logistic regression with enter method was used also to predict suicide attempts and affective/anxiety disorders (additional analysis for dissertation), and sleep duration (**Paper V**). Calculations were made with SPSS.16.0. (SPSS Inc., Chicago, IL, USA) with data in **Papers II, III and IV**.

Mixed-effects analysis of variance, e.g., multilevel models (MLM) was used for analysing longitudinal effects of the *NPSR1* genotype, age, sex, SLE and family relations on personality and ADHD scores (**Paper I**); and on bedtime and sleep quality, considering covariates BMI and month filling the questionnaire for **Paper V**. MLM longitudinal analyses were performed with SAS 9.1.3. (SAS Institute Inc., Cary, NC, USA) using PROC MIXED. Contrasts were calculated for significant MLM effects. Results from MLM were reported in the form of t-statistic, raw *p*-value and confidence intervals (CI) for group means.

4. RESULTS AND DISCUSSION

4.1. The *NPSR1* A/T genotype distribution in published studies

The distribution of the *NPSR1* A/T genotype in studies published so far reveals significant differences. Some of these reflect differences between ethnic groups (Table 1). The TT genotype was most common in a Japanese sample and least common in German samples. In Estonia, the genotype frequencies yield a rather balanced distribution of both alleles. Psychiatric "risk genotypes", such as the S allele of the 5-HTTLPR and the Met allele of *BDNF* Val66Met, are known to be less frequent in Caucasians but more common in Asian people (e.g., Goldman et al. 2010; Pivac et al. 2009), Estonian samples being observed as typical Caucasian groups. In case of *NPSR1,* Estonians appear to position between Japanese and the German Caucasian samples. However, we cannot make a strict conclusion about the allelic variation of the *NPSR1* rs324981 in this regard as we know of the distribution in only one Asian sample, and there is non-negligible variation between German samples.

Table 1 The *NPSR1* rs324981 genotype distribution in primarily non-clinical samples; Chi-square test for differences in ethnic groups.

The sample	*NPSR1* genotype				
	AA	AT	TT	Total N	Difference from ... sample
Estonian total	608 (25.3%)	1216 (50.6%)	578 (24.1%)	2402	Japanese: X^2=15.5, p<0.001; German: X^2=13.3, p=0.001
Driving School	191 (24.7%)	400 (51.7%)	182 (23.5%)	773	
Males	84 (26.4%)	164 (51.6%)	70 (22.0%)	318	
Females	107 (23.5%)	236 (51.9%)	112 (24.6%)	455	
Car Drivers Males only	123 (25.1%)	247 (50.3%)	121 (24.6%)	491	
ECPBHS	294 (25.8%)	569 (50.0%)	275 (24.2%)	1138	
Males	136 (26.3%)	258 (49.8%)	124 (23.9%)	518	
Females	158 (25.5%)	311 (50.2%)	151 (24.4%)	620	

Table 1. Continuation

The sample	*NPSR1* genotype				
	AA	AT	TT	Total N	Difference from ... sample
Japanese: Okamura et al. (2007) controls	52 (21%)	106 (43%)	87 (36%)	245	Estonian: X^2=15.5, p<0.001; German: X^2=28.2, p<0.001
German total	641 (29.7%)	1068 (49.4%)	451 (20.9%)	2160	Japanese: X^2=28.2, p<0.001; Estonian: X^2=13.3, p=0.001
Domschke et al. (2011) controls	264 (34.0%)	366 (47.2%)	146 (18.8%)	776	
Klauke et al. (2014) controls	131 (27.6%)	245 (51.6%)	99 (20.8%)	475	
Kumsta et al. (2013)	56 (28.6%)	98 (50.0%)	42 (21.4%)	196	
Lennertz et al. (2012) controls	190 (26.6%)	359 (50.4%)	164 (23.0%)	713	

4.2. *NPSR1* effects on personality, impulsivity and ADHD-related symptoms (Papers I and IV)

We first examined whether the functional *NPSR1* rs324981 polymorphism influences personality, impulsivity and hyperactive/inattentive behaviours, and whether any eventual associations depend on age, sex, stressful life events and family relations (**Paper I**).

In animal studies, higher NPS-ergic activity that corresponds to *NPSR1* rs324981 T-allele in humans produces arousal concurrently with anxiolytic effects (e.g., Pape et al. 2010; Xu et al. 2004) that is a rather unusual profile of action as pharmacological compounds that reduce anxiety also reduce arousal and activity. Human studies have so far been in agreement on that T-allele is associated with arousal (e.g., Dannlowski et al. 2011; Domschke et al. 2011) but have also suggested, contrary to animal findings, that T-allele is associated with higher anxiety as well (Dannlowski et al. 2011; Domschke et al. 2011; Donner et al. 2010; Klauke et al. 2014; Okamura et al. 2007; Raczka et al. 2010).

The key findings in **the ECPBHS sample** were: 1) subjects with the *NPSR1* TT genotype had the highest level of impulsive tendencies; 2) subjects with the TT genotype were most sensitive to SLE; and 3) subjects with the AA genotype were, instead, more sensitive to the family environment. The simplified

overview of these results is presented in Figure 1. The data have been derived from three measurement waves of two separate birth cohorts of ECPBHS. The effects on ADHD-related symptoms were apparent in both cohorts, although more evident in the younger cohort, whereas the main effects of *NPSR1* and interaction effect with sex on personality and impulsivity were observed primarily in the older cohort due to differences at age 18, and even more prominently, in young adulthood at age 25. This is plausible, as a part of ADHD symptoms are known to decrease with adolescence and adulthood, while stable personality is formed in early adulthood. *NPSR1* interaction effects with SLEs and family relations on personality, including impulsivity, were observed in both cohorts.

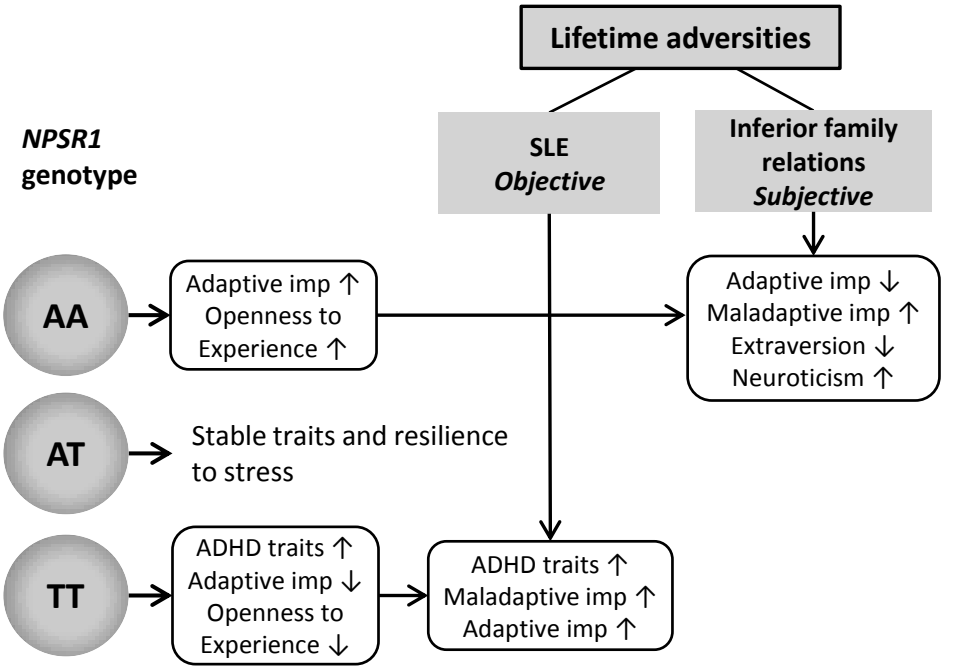

Figure 1 Simplified overview of the results of the *NPSR1* effects and interactions with SLE and family relations in the ECPBHS (**Paper I**). Imp – impulsivity.

The results described in **Paper IV** provide further evidence for the association of *NPSR1* A/T polymorphism with impulsivity and hyperactivity. These analyses used entirely independent samples. Again, subjects, especially males, carrying the T-allele had higher scores of both impulsivity and ADHD-related symptoms. However, we had no information about exposure to environmental adversities for these samples and could not check for G × E effects.

Impulsive action

The *NPSR1* A/T polymorphism influenced impulsivity as measured by the AMIS scale that enables to differentiate between adaptive and maladaptive types of impulsivity (Dickman 1990; Paaver et al. 2006) (**Papers I and IV**). We have also measured impulsivity with Barratt Impulsivity Scale but no differences between *NPSR1* genotypes were observed in any of the samples. This obviously suggests that the impulsivity constructs of Barratt significantly differ from others with regard to neurobiology that is NPS-related, and informs further studies on the brain circuits important for impulse control.

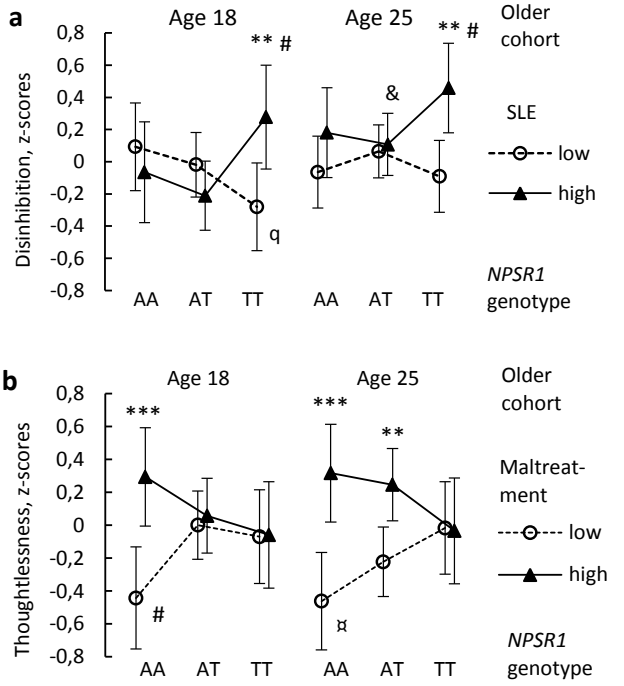

Figure 2 *NPSR1* × environment effects on maladaptive impulsivity in the ECPBHS. **(a)** *NPSR1* × SLE × Age effects on Disinhibition, $t(1, 279)=2.70$, $p=0.007$. (**b)** *NPSR1* × Maltreatment effect on Thoughtlessness, $t(1, 366)=–2.96$, $p=0.003$, at age 18 $F(2, 311)=0.13$, $p=0.877$, $\eta^2=0.001$; at age 18 $F(2, 247)=3.49$, $p=0.032$, $\eta^2=0.018$. ** $p<0.01$, *** $p<0.001$, difference from different environment, same genotype and age; $^{\#}$ $p<0.05$, difference from different allele, same age and environment; $^{\&}$ $p<0.05$, difference from age 18, same genotype and environment; ^{q} $p=0.06$, difference from A-allele carriers, same environment and age; $^{¤}$ $p<0.05$, difference from TT genotype, same environment and age.

In the EPSTB sample, male T-allele carriers had higher impulsivity scores; however, in Car Drivers, it was higher adaptive impulsivity, whilst in the Driving School sample, it was higher maladaptive impulsivity (**Paper IV)**. It is important to recognize, that both types of impulsivities are sensitive to G × E effects (**Paper I)**. TT homozygotes had significantly higher impulsivity in case of high SLE that hypothetically may indicate the rise of activity in response to stress (e.g., Figure 2a). This fits well with the modest effects of *NPSR1* on ADHD related behaviour as rated by teachers, where the TT males had more symptoms (Figure 3), and SLE further increased the scores.

In ECPBHS sample, subjects with the AA genotype, especially males, had the steepest elevation in Adaptive impulsivity fom age 18 to 25. AA homozygotes also gained the most from favourable family relations by reacting with elevated Adaptive impulsivity and Extraversion, and with lowered Thoughtlessness and Neuroticism (Figures 2b and 4). This may be because supportive environment lowers anxiety. On the other hand, unfavourable family environment increased Thoughtlessness and Neuroticism and lowered Extraversion in subjects with AA genotype that may indicate the harmful effects of anxiety (Figures 2b and 4). So the *NPSR1* effects on both types of impulsivities and personality may reflect changes in balance between activity/ arousal and anxiety. Such a notion appears to be consistent with animal studies where NPS-ergic neurotransmission concurrently promotes arousal and lowers anxiety (Lukas & Neumann 2012; Rizzi et al. 2008; Wegener et al. 2012; Xu et al. 2004).

We found no evidence for genotype differences in social deviance that was available for the Driving School sample only (for males, n=315, $F<0.01$, $p=0.979$, $\eta^2<0.001$; for females, n=449, $F=0.77$, $p=0.381$, $\eta^2=0.002$). While high impulsivity may lead to socially irresponsible behaviours, this appears not to be the case with *NPSR1*-related impulsivity. Nevertheless, we cannot rule out G × E effects on social deviance.

In both cohorts, males with the TT genotype of the *NPSR1* had more ADHD symptoms as observed by teachers (**Paper I**). Stressful life events further increased the ADHD scores in TT genotype. We also found that the TT genotype was overrepresented in the upper 5% of ADHD scores at age 15. The reason for failing to detect this at older age may be based on the higher dropout rate of the subjects with the extreme values of ADHD scores: while overall 94% of 15-years-old subjects from younger cohort participated in the next wave, it was only 63% of the extremely high ADHD score subjects.

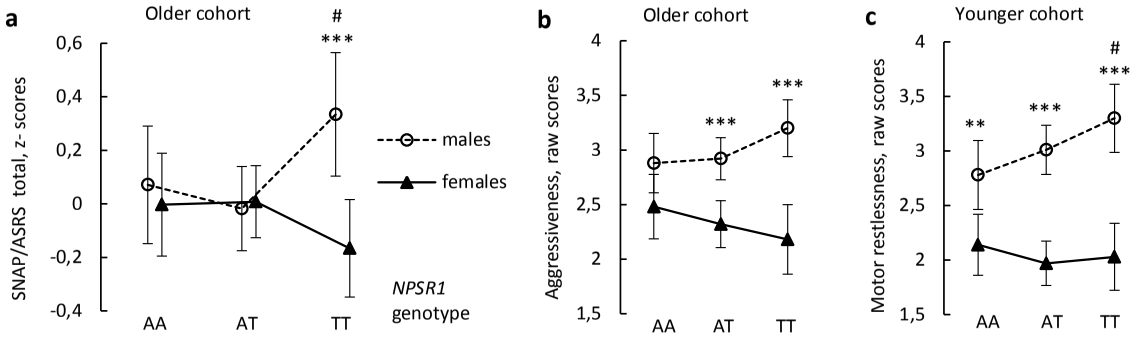

Figure 3 *NPSR1* × Sex effect on ADHD scores in the ECPBHS. (**a**) *NPSR1* × Sex effect on SNAP/ASRS total scores in the older cohort, $t(1, 498)=-1.97$, $p=0.049$. (**b**) *NPSR1* × Sex effect on Hyperactivity subscale Aggressivity in the older cohort, $t(1, 565)=-2.22$, $p=0.027$. (**c**) *NPSR1* × Sex effect on Motor Restlessness in the younger cohort, $t(1, 542)=1.97$, $p=0.049$. ** $p<0.01$, *** $p<0.001$, difference from females, same genotype; $^{\#}p<0.05$, difference from A-allele males.

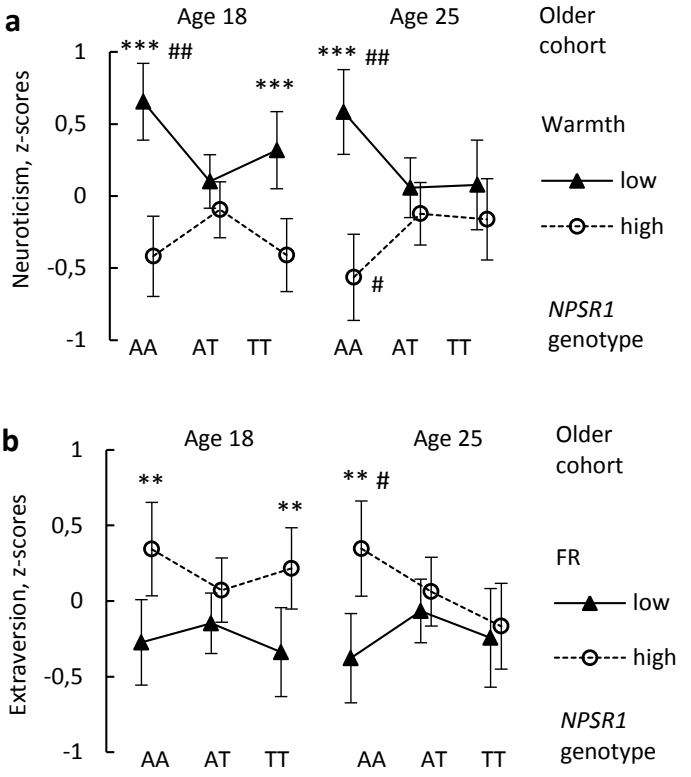

Figure 4 *NPSR1* × family environment × Age effects on personality in the ECPBHS. **(a)** *NPSR1* × Warmth × Age effect on Neuroticism, $t(1, 324)=2.53$, $p=0.012$. (**b)** *NPSR1* × Family relations × Age effect on Extraversion, $t(1, 316)=2.02$, $p=0.044$. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, difference from different environment, same genotype and age; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, difference from T-allele, same environment and age.

As T-allele carriers, especially male TT homozygotes**,** expressed increased activity (**Papers I and IV)**, and higher arousal in the gain-of function T-allele carriers has been established previously (e.g., Domschke et al. 2011), *NPSR1* appears as a strong ADHD candidate gene, supported by animal models (Reinscheid 2008). This is also consistent with the results of the impulsivity-related role of the *NPSR1* (**Papers I and IV)**. Both ADHD patients and individuals with above-average impulsivity are highly responsive to situations and easily aroused emotionally, which is in accordance with the enhanced reactivity to emotional stimuli in the T-allele carriers (Raczka et al. 2010; Dannlowski et al. 2011). Nevertheless, Okamura et al. (2007) reported no association of *NPSR1* A/T polymorphism with ADHD. Neither have searches for ADHD candidate genes pointed towards *NPSR1* or its location on chromosome 7p14.3 (e.g., Banaschewski et al. 2010; Lesch et al. 2008; Neale et al.

2008). So the NPS receptor may rather contribute to ADHD-like behaviour dimensionally and through impulsivity and arousal, effects that become evident in non-clinical samples and may have public health relevance in general population.

In response to centrally administered NPS in rodent models, the increased locomotor activity is accompanied by wakefulness and reduced sleep (Rizzi et al. 2008; Xu et al. 2004; Zhao et al. 2012). In children, hyperactive and inattentive symptoms are also related to short sleep duration, so insufficient sleep and fighting drowsiness may be causally related to these symptoms (Paavonen et al. 2009). NPS-ergic neurotransmission has a direct effect on the HPA axis and contributes to the modulation of arousal and anxiety by regulating the release of CRF (Paneda et al. 2009; Smith et al. 2006; Zhao et al. 2012). Speculatively, the human *NPSR1* A/T polymorphism may reflect a population-level balancing mechanism for fighting drowsiness and low cortical activity, and boosting arousal, which is observable to the largest extent in males with the TT genotype (**Paper I)**, already mimicking the ADHD symptoms. In the EPSTB samples, high impulsivity and hyperactivity were present not exclusively in TT homozygotes, but T-allele carriers (**Paper IV)**. We thus may suggest that in general population, subjects with the T-allele have higher-functioning arousal/anxiety system that does not lead automatically to anxiety-related psychopathology.

Stressful life events and family relations

There were no differences in evaluating family environment or the occurrence of stressful life events by *NPSR1* in ECPBHS subjects. However, our findings suggest that both AA and TT *NPSR1* homozygotes respond to stress more than heterozygotes, but the vulnerabilities differ: AA responded more to family environment, and TT mainly to stressful life events **(Paper I)**. The fact that the AA genotype (the genotype leading to the least active NPSR receptor-mediated signal transduction) was reactive to stress may demonstrate that elevation of anxiety in adverse family environment is not well compensated for by NPS if the receptors are less sensitive. This would be consistent with animal studies where *NPSR1* knockout mice expressed increased anxiety-like behaviour (Duangdao et al. 2009) and administration of NPS acted like an anxiolytic (Jüngling et al. 2008; Lukas & Neumann 2012; Wegener et al. 2012; Xu et al. 2004). Otherwise, subjects with TT and not AA genotype were sensitive to adverse life events – they had significantly higher scores of impulsivity, inattention and motor restlessness (the latter in males only).

The sensitivity to environment of the T-allele carriers is in accordance with previous human studies where all the presumed adverse arousal and anxiety-related psychiatric effects were limited to T-allele carriers. Similarly, in the single previous G × E study (Klauke et al. 2014), *NPSR1* TT genotype subjects were more sensitive to adverse life events, reporting higher anxiety sensitivity. NPS activates HPA axis (Paneda et al. 2009; Smith et al. 2006; Zhao et al.

2012), and enhanced acute stress response was recently detected in T-allele carriers (Kumsta et al. 2013). Importantly, however, all this is not easily compatible with animal studies: a simple deduction from the facts that NPS administration evokes anxiolytic effects in rodents, attenuates the expression of contextual fear (Meis et al. 2008) and facilitates fear extinction (Jüngling et al. 2008) would be that in humans the T-allele carriers, especially the TT homozygotes, have better functioning in response to stress compared to those with A-alleles. Translating our findings that both AA and TT homozygotes are less resilient to environmental adversities back to animal studies, one could expect divergent effects of NPS on anxiety-related behaviour in radically different experimental designs. For another neuropeptide, cholecystokinin, this has been reported (see Harro 2006 for review).

Indeed, what is different in the two measures of environmental adversities requires further analysis. Conceivably, less warm family environment overall and specific adverse life events are different stressors although they correlate moderately. In our sample, these two types of adversities had quite similar effects on personality, impulsivity and ADHD-related symptoms. A working hypothesis is that the key is the relative subjectivity of the evaluation: while life events were mostly reported as having occurred or not (e.g., death of a close relative), the family environment assessment was quantitative and based on perceptive evaluation. Whether objective or subjective indicators of environmental stress evaluations are used does affect the outcome (Monroe 2008).

Self-reports of adverse life events may underestimate their occurrence. In this study such a bias could affect the results only if underreporting were genotype-dependent. This nevertheless cannot be excluded and merits attention in future studies.

It has been reported that *NPSR1* A/T polymorphism influences emotional evaluation, and T-allele carriers react more strongly to fear-related emotional stimuli possibly caused by higher arousal of T-allele (Dannlowski et al. 2011; Domschke et al. 2011; Raczka et al. 2010). These findings guide the thinking to explanation that the higher response to SLE of subjects with two T alleles could be related to arousal mechanisms that are separate from the neurobiology of anxiety, and the reaction of AA genotype on family environment in **Paper I** must be anxiety-related as derived from animal studies. Without knowing the exact neural mechanisms yet, we can however conclude that a double dissociation of perception of family environment (as measured by the scale we used) and adverse life events occurs in *NPSR1* AA and TT homozygotes, resulting in distinct G x E outcomes in personality, impulsivity and ADHD symptoms.

The effects of the *NPSR1* AA genotype on personality and impulsivity were advantageous in case of favourable family environment, and more so for men (**Paper I**). Nevertheless, the sensitivity to family environment of the subjects with AA genotype led to detrimental changes as reflected e.g., in scores of Neuroticism in case of adverse family relations.

4.3. *NPSR1* × environment effects: contribution to affective and anxiety disorders, and suicidal behaviour (Paper II)

Considering the evidence of the role of NPS-ergic neurotransmission in the regulation of emotional processing (Dannlowski et al. 2011; Domschke et al. 2011; Klauke et al. 2014; Raczka et al. 2010; Tupak et al. 2013), panic disorder (Domschke et al. 2011; Donner et al. 2010; Okamura et al. 2007), and *NPSR1* interactions with the environment in shaping traits **(Paper I**) that predict anxiety and affective disorders (e.g., Lönnqvist et al. 2009), we aimed to clarify the role of *NPSR1* A/T polymorphism for anxiety and mood disorders in the general population, also taking into account the sensitivity of the genotype to environmental factors.

We found that in females with the functionally least active *NPSR1* AA genotype who had experienced adverse family environment, lifetime affective/ anxiety disorders were more prevalent by age 25 (Figure 5a). This higher prevalence of psychiatric disorders was accompanied by high anxiety and low self-esteem (Figures 6 and 7), traits that both predict affective disorders and suicidal behaviour (Domschke & Reif 2012; Mann et al. 2004; Moffitt et al. 2007; Wasserman 2001; Wild et al. 2004). Indeed, G × E interaction effects had been present already in adolescence as reflected in higher depressiveness and anxiety, lower self-esteem and higher frequency of suicide attempts in AA homozygotes (Figure 5b).

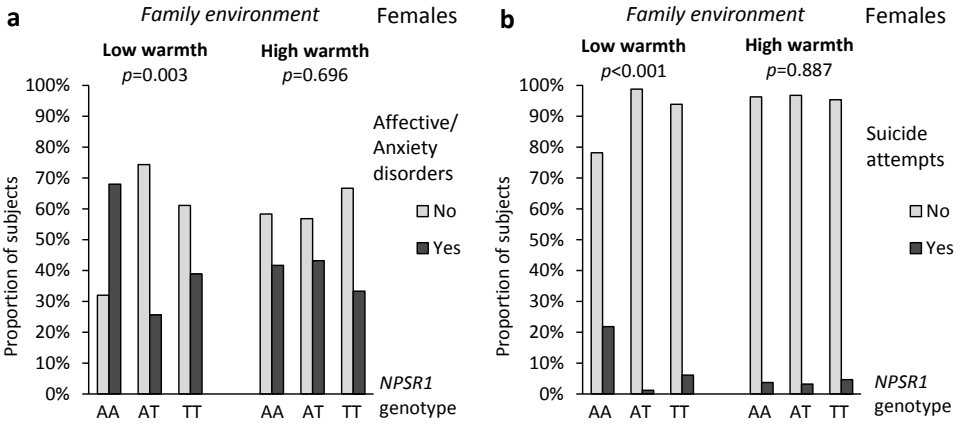

Figure 5 *NPSR1* × Warmth in the family association with affective/anxiety disorders and suicide attempts in females of the ECPBHS. **(a)** Affective/anxiety disorders in the older cohort by age 25, n=179; **(b)** Suicide attempts in both cohorts by age 18, n=461.

It is also known that the risk for suicidal behaviour is heightened by impulsivity (Fawcett 2001; Nock et al. 2008), and the risk for anxiety/affective disorders is increased by neuroticism and lowered by extraversion (Bienvenu et al. 2004; Kotov et al. 2010). In accordance with this, subjects with the AA genotype who experienced adverse family environment developed high maladaptive impulsivity and neuroticism, and low extraversion (**Paper I)** that could contribute to suicide attempts and disorders. Hence the question emerges, whether the association of the *NPSR1* with suicidal behaviour is mediated by these personality traits or the disorders. Additional analysis to predict suicide attempts with the genotype and predictors from earlier age revealed, that besides *NPSR1* AA genotype, female sex and higher SLE, suicide attempts were only predicted by lower extraversion reported three years earlier, and not neuroticism (Table 2). The *NPSR1* genotype was a stronger predictor of suicide attempts than extraversion. Similar results were obtained when predicting affective/anxiety disorders (not shown in detail): significant predictors from age 15 were female gender (OR=3.15 [1.26–7.90], p=0.014), the AA genotype (OR=2.7 [1.20–6.12], p=0.019) and higher SLE (OR=2.34 [1.04–5.30], p=0.041), while personality did not contribute significantly. These associations held and were even stronger when females were analysed separately. So, *NPSR1* appears as an independent predictor of both suicide attempts and affective/anxiety disorders, and contributes to the development of personality and impulsivity described in **Paper I**. Thus, the accumulation of harmful traits in that are magnified by adverse environment possibly lead to suicidal behaviour and ideation, and affective/anxiety disorders in females with the AA genotype. These phenotypes are correlated with each other and are likely to share underlying biology; nevertheless, they still are different dimensions with their own mechanisms of development; to which extent these are interwoven, requires further study. Thus, the *NPSR1* genotype appears to represent a basic system involved in vulnerability to emotion dysregulation.

The contribution of the *NPSR1* AA genotype to affective/anxiety disorders is compatible with results of animal studies where less active NPS-ergic neurotransmission is associated with anxiety-related behaviour (Lukas & Neumann 2012; Rizzi et al. 2008; Wegener et al. 2012; Xu et al. 2004). In addition, very recent evidence points to the involvement of A-allele in anxiety-related psychopathology in humans: Subjects with the AA genotype were reported to have higher trait anxiety in one study (Glotzbach-Schoon et al. 2013); in others, the A-allele carriers had more likely early onset obsessive-compulsive disorder (Lennertz et al. 2013), and were over-represented among patients with schizophrenia (Lennertz et al. 2012) which is not surprising as anxiety may be the core symptom dimension in schizophrenia (reviewed by Muller et al. 2004).

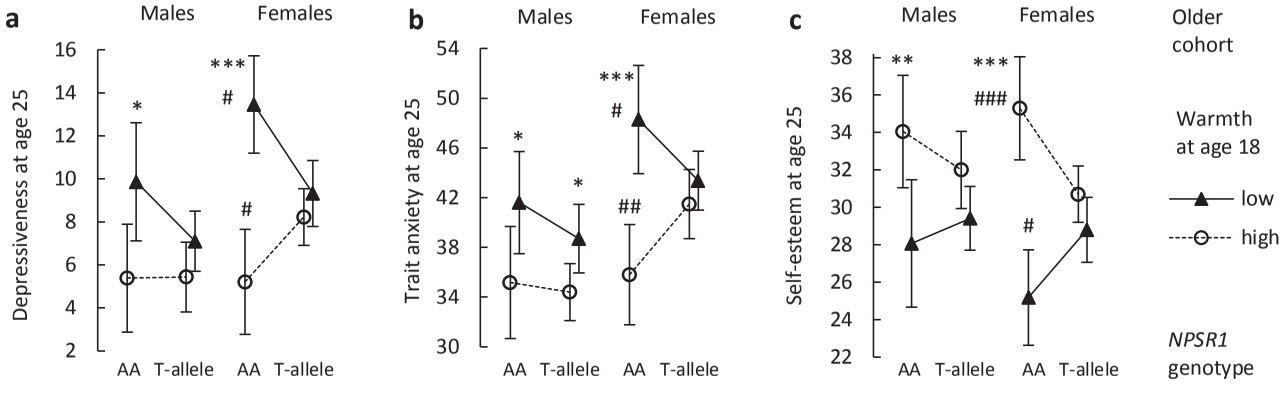

Figure 6 *NPSR1* × Warmth effects on Depressiveness, Trait Anxiety and Self-esteem, by sex in the older cohort of the ECPBHS at age 25. **(a)** Depressiveness; in males F(1, 130)=1.69, p=0.197, η^2=0.013; in females F(1, 192)=13.1, p<0.001, η^2=0.064 (the overall effect F(1, 326)=16.1, p<0.001, η^2=0.047). **(b)** Trait anxiety; in males: F(1, 127)=0.35, p=0.553, η^2=0.003; in females: F(1, 188)=9.01, p=0.003, η^2=0.046 (the overall effect F(1, 319)=10.6, p=0.001, η^2=0.032). **(c)** Self-esteem; in males F(1, 122)=1.63, p=0.204, η^2=0.013; in females F(1, 188)=13.5, p<0.001, η^2=0.067 (the overall effect F(1, 314)=15.1, p<0.001, η^2=0.046). * p<0.05, ** p<0.01, *** p<0.001, difference from different environment, same genotype; $^{\#}$ p<0.05, $^{\#\#}$ p<0.01, $^{\#\#\#}$ p<0.001, difference from T-allele carriers, same environment and sex.

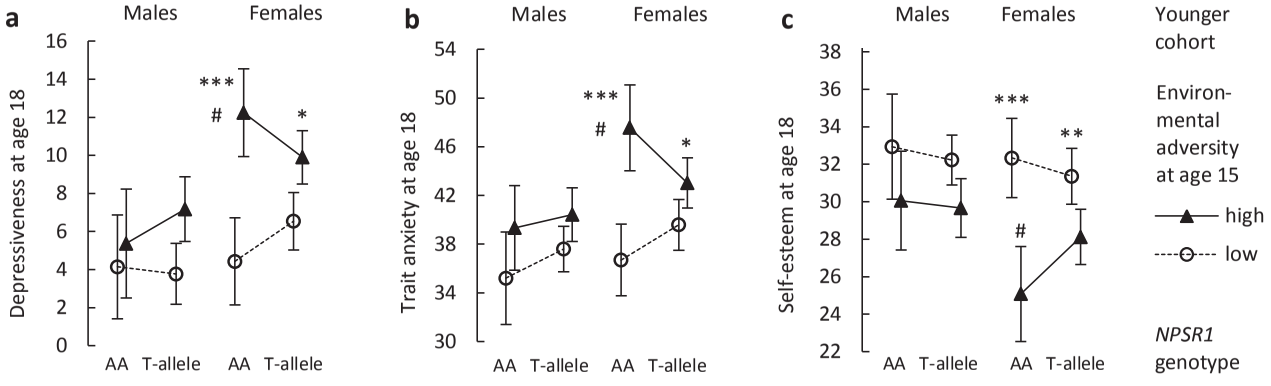

Figure 7 *NPSR1* × Environment effects on Depressiveness, Trait Anxiety and Self-esteem by sex in the younger cohort of the ECPBHS at age 18. **(a)** Depressiveness, *NPSR1* × SLE 15y; in males F(1, 179)=0.71, p=0.400, η^2=0.004; in females F(1, 218)=4.71, p=0.031, η^2=0.021 (the overall effect F(1, 401)=1.68, p=0.196, η^2=0.004). **(b)** Trait anxiety, *NPSR1* × Maltreatment; in males: F(1, 136)=0.19, p=0.662, η^2=0.001; in females: F(1, 203)=7.22, p=0.008, η^2=0.034 (the overall effect F(1, 343)=4.72, p=0.030, η^2=0.014). **(c)** Self-esteem, *NPSR1* × Maltreatment; in males F(1, 143)=0.02, p=0.886, η^2<0.001; in females F(1, 204)=4.11, p=0.044, η^2=0.020 (the overall effect F(1, 351)=2.56, p=0.110, η^2=0.007). $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001, difference from different environment, same genotype; $^{\#}p$<0.05, difference from T-allele carriers, same environment and sex.

Table 2 Predicting suicide attempts by personality reported 3 years earlier in combined cohorts of the ECPBHS, results from simultaneous logistic regression. Bold – significant effect.

Predictor	Wald statistic	*p*	Exp(β) [95% confidence interval]
Sex (females)	**8.53**	**0.003**	**5.61 [1.76–17.8]**
***NPSR1* (AA** vs T-allele**)**	**6.58**	**0.010**	**2.84 [1.28–6.32]**
Neuroticism	0.03	0.863	1.04 [0.66–1.63]
Extraversion	**5.40**	**0.020**	**0.60 [0.39–0.92]**
Openness to experience	0.32	0.571	0.86 [0.58–1.35]
Agreeableness	0.43	0.514	0.87 [0.58–1.32]
Conscientiousness	0.15	0.702	0.91 [0.56–1.48]
SLE at age 15 (high)	**7.12**	**0.008**	**3.11 [1.35–7.15]**
Constant	19.7	<0.001	

As mentioned above, these new findings of ours, and others, are in an apparent contrast with the original suggestion that the *NPSR1* T-allele is associated with panic disorder (Domschke et al. 2011; Donner et al. 2010; Okamura et al. 2007) that belongs to the group of anxiety disorders. To reconcile the inconsistency with animal studies that rather suggest the A-allele as anxiety-related, the *NPSR1*-associated panic disorder was hypothesized to arise from arousal mechanisms, not anxiety (Domschke et al. 2011; Okamura et al. 2007), as NPS-ergic transmission is associated with higher arousal/activity in both animal (e.g., Guerrini et al. 2010; Smith et al. 2006) and human studies (e.g., Domschke et al. 2011; **Paper I**). The biological differentiation of panic disorder from anxiety would be in accordance with Panksepp`s theory of basic limbic emotional action systems, the primary sources of fear response and panic being in neurobiologically independent domains (Panksepp 1998; Panksepp 2005). Higher arousal does not have to lead to anxiety-related psychopathology (panic disorder set aside), given that NPS has the unique pattern of effects in animals, being concurrently stimulant and anxiolytic (e.g., Pape et al. 2010). Instead, the higher arousal and reactivity of the amygdala / dorsal anterior cingulate cortex / dorsomedial PFC system (Dannlowski et al. 2011; Raczka et al. 2010) as well as the less prominent cortical activation in response to emotional stimuli (Tupak et al. 2013) in T-allele carriers could indicate decreased inhibitory control (Kim & Lee 2011). Impulsive action that is more characteristic to subjects with the TT genotype (**Papers I and IV**) may thus contribute rather specifically to e.g., panic disorder and bipolar disorder. Indeed, lower activity of dorsolateral PFC is observed in panic disorder and post-traumatic stress disorder, but in case of anxiety disorders that involve worry and rumination (recursive self-focused thinking) such as generalized anxiety and obsessive-compulsive disorder, the PFC is characteristically more active (Berkowitz et al. 2007; Strawn et al.

2012). Also rumination in depression is associated with increased activation in limbic areas and in dorsolateral PFC (Cooney et al. 2010). Consistent with this notion, AA subjects have high activity in dorsolateral PFC (Domschke et al. 2011; Tupak et al. 2013). Thus, increased dorsolateral PFC activity in subjects with the AA genotype may not only be an indicator for stronger amygdala input suppression but also for repetitive self-focused thinking. In addition, rostral dorsomedial PFC activity is reduced in depressed females in response to negative stimuli (Moses-Kolko et al. 2010) similarly to subjects with the AA genotype in the Raczka et al. (2010) study. Repetitive thoughts may also represent a constructive form of cognitive coping strategies like planning, problem solving and rehearsal (Watkins 2008). In line with this, subjects with the AA genotype appear as being able to take advantage of favourable environments, as they had on average the highest level of self-esteem, elevated extraversion and adaptive impulsivity as well as the lowest anxiety and neuroticism (**Papers I and II**).

Essentially in consistency with animal studies where NPS has influenced the function of the HPA axis (Paneda et al. 2009; Smith et al. 2006), male T-allele carriers (no females were tested) had larger salivary cortisol responses to social stress in laboratory (Kumsta et al. 2013). The authors concluded that the T-allele represents one vulnerability factor to stress- and anxiety-related disorders if carriers were subjected to chronic stress. Again, this would however apparently contradict animal studies where higher NPS activity, that has stronger influence on the HPA axis, is simultaneously arousal-promoting and anxiolytic (e.g. Pape et al. 2010; Smith et al. 2006; Xu et al. 2004), and facilitates fear extinction (Han et al. 2013; Lukas & Neumann, 2012; Okamura et al. 2011). However, there is substantial difference between acute and chronically active stress response, with the first being adaptive/mobilising and the latter possibly having detrimental consequences on health (Chrousos 2009; Luine 2007; Nugent et al. 2011). So cortisol measurement one hour after social stress test as in Kumsta et al. (2013) provides no sufficient information to conclude on long-term effects of *NPSR1* on neuroendocrine and stress response. Evidence from **Paper I** agrees that T-allele carriers do respond to SLEs with maladaptive traits (higher impulsivity and hyperactivity) but these are AA homozygotes instead who react to adverse family environment with persistent changes in maladaptive traits (**Papers I and II**) that could accumulate and convert into affective/anxiety disorders (**Paper II**) that quite obviously do not develop overnight (Harro and Oreland 2001).

The effects of *NPSR1* as described in **Paper II** were generally female-dominant. Sex-dependent effects of *NPSR1* were found also by Dannlowski et al. (2011), Domschke et al. (2011), Okamura et al. (2007), and in studies leading to **Paper I**. The biological underpinnings behind the relative sex-specificity of the *NPSR1* associations could possibly be explained by the action of NPS on monoaminergic system (Didonet et al. 2014; Raiteri et al. 2009; Si et al. 2010) and on CRF release (Paneda et al. 2009; Smith et al. 2006). Sex-

specific neurochemical changes have been documented both in monoaminergic system (e.g., Jovanovic et al. 2008; Mällo et al. 2009; Van den Hove et al. 2014) and CRF function (Bangasser et al. 2010), and these differences are thought to explain sex differences in the prevalence of affective and anxiety disorders.

In conclusion, our findings suggest that the *NPSR1* A/T polymorphism is associated with affective and anxiety disorders and suicidal behaviour in a sex- and environment-dependent manner. It appears that in females the AA genotype and adverse family relations could lead to affective dysregulation, suicide attempts and internalizing psychopathology.

4.4. *NPSR1* × environment effects: contribution to alcohol consumption and alcohol use disorders (Paper III)

Given that *NPSR1* influences traits that are associated with substance use (**Papers I, II and IV**), and the involvement of the NPS system in addictive behaviours in rodent studies (reviewed by Cannella et al. 2013), we hypothetised that the *NPSR1* A/T polymorphism may contribute in a complex, certainly sex-dependent manner to alcohol use disorders (AUD) and alcohol use.

We indeed found that the associations of the *NPSR1* genotype with alcohol use and AUD were significant but differed by sex. In females, both AUD and excessive alcohol use were more prevalent in subjects with at least one A-allele. In contrast, AUD in males was more prevalent in carriers of the high-activity related T-allele, and especially so if the subjects had been exposed to environmental adversity in adolescence. Thus, male T-allele carriers had consumed alcohol more excessively in adolescence. It was surprising to find that the risk allele for higher alcohol use at age 25 was the A-allele in both females and males. This may relate to distinct pathways to higher alcohol use, as discussed below.

After testing the associations with personality traits that were found to be associated with *NPSR1* (**Paper I**) and are known to affect alcohol use, most of the genotype effects become weaker or disappeared. This suggests that *NPSR1* effects on AUD and alcohol use are at least partly mediated by personality.

The sex and time dependent nature of *NPSR1* associations with AUD and alcohol use are in line with the overall personality and impulsivity regulating role of *NPSR1* as found in studies described in **Papers I, II and IV**. Again, the *NPSR1* sex-specific effects could be explained by the NPS system interactions with monoaminergic systems (Didonet et al 2014; Mochizuki et al. 2010; Raiteri et al. 2009; Si et al. 2010) and HPA function (Cannella et al. 2009; Jüngling et al. 2012; Smith et al. 2006) that are known to play role in sex differences both in stress responses (Bangasser et al. 2010) but also in alcohol

abuse (reviewed by Witt 2007). We have presented data-driven developmental pathways to AUD in males and females by *NPSR1* genotype in Figure 8.

Subjects with higher neuroticism and emotion dysregulation tend to use alcohol because of its acute anxiolytic effects (Bolton, Robinson & Sareen 2009; Chartier et al. 2010; Kushner et al. 2011). This may apply to female A-allele carriers of this study as the genotype effects on AUD and alcohol use were mostly explained by neuroticism. They also reported feeling more relaxed and happier when drinking compared to the T-allele carriers, and this may refer to the regulation of negative emotions by alcohol use. Moreover, female A-allele carriers in this sample were vulnerable for affective and anxiety disorders (**Paper II**) and all females with anxiety/affective disorders comorbid with AUD were A-allele carriers. The harmful use of alcohol and AUD is indeed common among individuals with affective and anxiety disorders (e.g., Bolton et al. 2009; Kleinberg, Aluoja & Vasar 2010; Kushner et al. 2011). As alcohol dependence is considered to be related to overall internalizing psychopathology load rather than to a particular disorder (Kushner et al. 2012), we suggest that in females the lower *NPS*-ergic activity bears a risk for affective and anxiety-related dysregulation that renders them vulnerable also to alcohol use.

Contrary to females, the risk allele for AUD in males was the T-allele. The likelihood for the disorder in T-allele carriers was even higher if adverse environmental factors were accounted for. The pattern of more excessive alcohol use of male T-allele carriers was observed already at age 15. We have shown that subjects with the T-allele are more impulsive and hyperactive (**Papers I and IV**), and sensitive to stressful life events that increase disinhibition (**Paper I**). Early-age initiation of alcohol consumption and poor impulse control, which are often related, are both risk factors for developing substance use disorders (Diemen et al. 2008; Iacono et al. 2003). So development of alcohol addiction in males with at least one T-allele may possibly depart from initial higher activity/impulsivity level: these subjects start with larger quantities of alcohol, then stressful life events further accentuate disinhibition that loosens the control over alcohol consumption and leads to alcohol abuse, allowing the drug to take control. This hypothesis is supported by significant association of extraversion, adaptive and maladaptive impulsivity, hyperactivity and stressful life events with alcohol use and AUD in this study. In adolescents and young adults, early stages of heavy alcohol use are characterised by impulsive drinking (Heilig & Koob 2007) where activity of the mesolimbic dopaminergic system plays a crucial role (reviewed by Vengeliene et al. 2008). As NPS stimulates dopaminergic activity (Didonet et al. 2014; Mochizuki et al. 2010; Si et al. 2010), males with the high-activity T-allele could be especially vulnerable to excessive alcohol use patterns in adolescence associated with high dopaminergic activity.

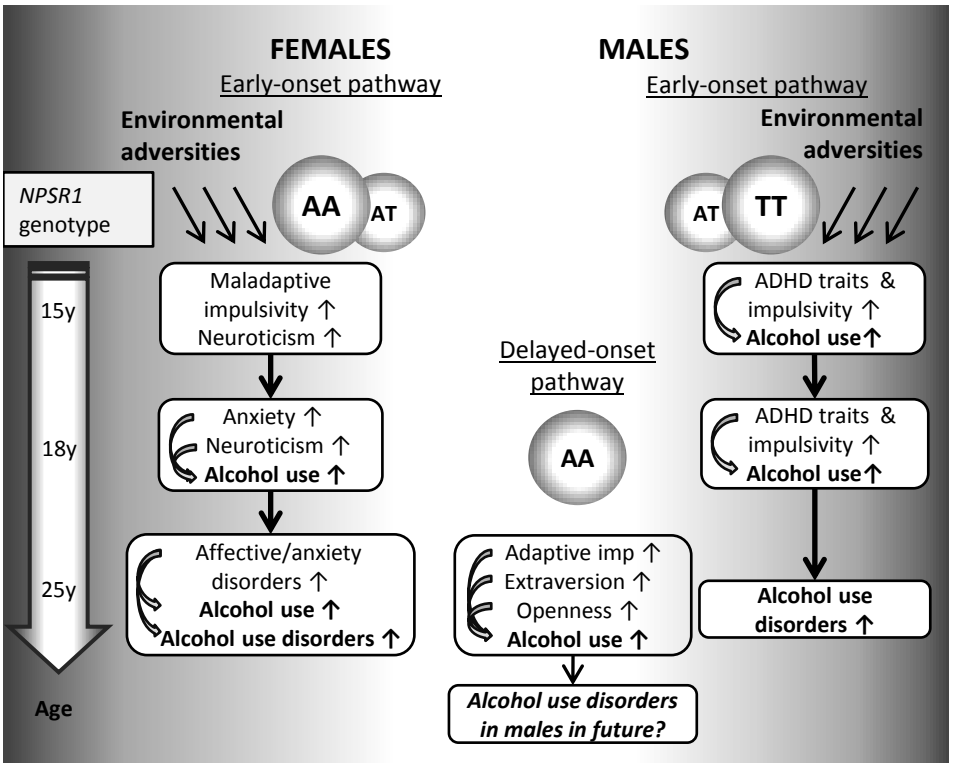

Figure 8 Proposed pathways to alcohol use disorders (AUD) in males and females by *NPSR1* genotype. In females, the lower *NPS*-ergic activity in A-allele carriers and especially in AA homozygotes bears a risk for affective and anxiety-related dysregulation already in adolescence (**Paper II**) that also renders them vulnerable to alcohol use. Consequently, some females carrying the A-allele develop AUD already in young adulthood. In males, an impulsivity-related early-onset pathway to AUD occurs in T-allele carriers and in particular in TT homozygotes: already in adolescence, they exhibit more ADHD symptoms and impulsivity (**Paper I**) that make them vulnerable to alcohol use, especially when experiencing adverse environment (**Paper I**). As a consequence, many males carrying the T-allele develop AUD by young adulthood. In males, a delayed-onset pathway to AUD is also suggested for the AA genotype: the increase of adaptive impulsivity, extraversion and openness to experience by age 25 in males with AA genotype (**Paper I**) can make them vulnerable to higher alcohol use. However, future studies have to reveal whether AUD will develop on this basis in male AA subjects in later age. ADHD traits – ADHD-related traits; imp – impulsivity; Openness – Openess to experience.

Interestingly, the risk allele for higher alcohol use was not T-allele anymore by young adulthood, and more alcohol was consumed by males with the AA genotype instead. This discrepancy can be explained by the significant progression of adaptive impulsivity from adolescence to age 25 in males with the AA genotype (**Paper I**) that is generally beneficial but is also associated with substance use, and possibly very much so in a country with a general high level of alcohol consumption. Indeed, AA genotype effects on alcohol use in males were explained by both high adaptive impulsivity and neuroticism. Again, future studies will have to test whether AUD will develop in male AA subjects in later age than in T-allele carriers.

4.5. Neuropeptide S receptor gene (*NPSR1*) A/T polymorphism and sleep (Paper V)

Consistent with animal studies where higher NPS-ergic activity is associated with reduced sleeping time and increased wakefulness (e.g., Xu et al. 2004; Zhao et al. 2012), the T-allele carriers had later bedtime in a study of circadian phenotypes by Gottlieb et al. (2007). However, subjects with the AA genotype had higher prevalence of affective/anxiety disorders (**Paper II**) and higher trait anxiety (**Paper II**; Glotzbach-Schoon et al. 2013) that go hand in hand with sleep problems (reviewed by Shochat et al. 2014). But sleep disturbances and shorter sleep duration are also associated with hyperactivity (Ganelin-Cohen & Ashkenasi 2013) that appears as characteristic to T-allele carriers (**Papers I and IV**). Except the GWAS quoted above (Gottlieb et al. 2007), no study has been published to date on *NPSR1* link to sleep difficulties.

In the present analysis, *NPSR1* rs324981 (A/T) polymorphism was associated with both bedtime and sleep duration, and also with sleep-related problems.

The *NPSR1* association with bedtime changed with age. While subjects with the AA genotype had the latest bedtime in childhood and middle adolescence, no differences in bedtime were observed at age 18, and in young adulthood at age 25, those were subjects with the TT genotype who had the latest bedtime. We also found age-dependency in effects of *NPSR1* on personality and alcohol use in these birth cohorts (**Papers I and III**) and this is not surprising as adolescence is a period with rapid changes in the CNS (reviewed by Casey et al. 2008). As mentioned above, the latest bedtime in adult TT homozygotes was previously reported by Gottlieb et al. (2007), and these results are consistent with animal studies where higher NPS-ergic activity is associated with reduced sleep (Camarda et al. 2009; Hirota et al. 2009; Jüngling et al. 2008; Rizzi et al. 2008; Smith et al. 2006; Xu et al. 2004).

Reduction of sleep time by *NPSR1* in the ECPBHS sample was observed only when environmental factors were accounted for, and only in adulthood. At age 25, the latest time point available, subjects with the TT genotype slept less if they had reported higher number of stressful life events (SLE) in adolescence;

and subjects with the AA genotype had shorter sleeping time if they had experienced adverse family environment. G × E effects were present also in case of sleep-related problems: again more problems were reported by subjects with the AA genotype in case of adverse family environment, and by subjects with the TT in case of high SLE. One has to recall that the preferential sensitivity of TT genotype to SLEs and AA to family environment was also found in associations of *NPSR1* with personality and hyperactivity (**Paper I**). Consistently, subjects with the TT genotype reported higher anxiety sensitivity in Klauke et al. (2014) if they had experienced higher number of adverse life events. In ECPBHS, subjects with the AA genotype more often reported suicide attempts and were diagnosed with an affective/anxiety disorder in case of adverse family environment (**Paper II**).

NPSR1 had effects on sleep-related difficulties in both birth cohorts of the ECPBHS but the effects were different. In the older cohort, subjects with the AA genotype had the highest number of sleep-related problems whereas subjects with the TT genotype had the least problems (Figure 9). However, in AA homozygotes the high frequency of sleep problems depended on adverse family environment in adolescence. In the younger cohort, similarly, higher scores in AA homozygotes in case of less warm family environment were observed, but the association emerged at later age. Higher frequency of sleep-related problems in subjects with the AA genotype, especially when experiencing adverse family environment, was expected as we had found that subjects with the AA genotype had higher prevalence of affective/anxiety disorders, the highest frequency of suicide attempts and the highest scores of depressiveness and anxiety, if reporting adverse family environment in late adolescence (**Paper II**). In addition, AA homozygotes had higher prevalence of

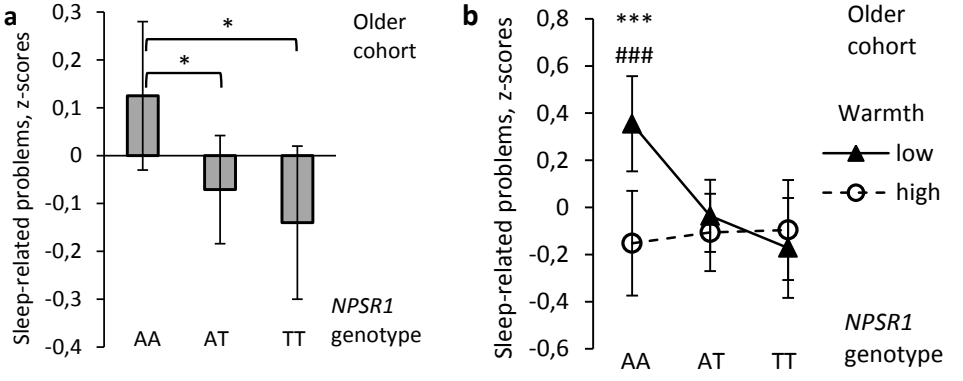

Figure 9 *NPSR1* effects in sleep-related difficulties in the older cohort of the ECPBHS. (a) *NPSR1* main effect, t=–2.36, p=0.019. (b) *NPSR1* × warmth effect, t=2.48, p=0.014. * p<0.05, *** p<0.001, differences from AT or TT genotype, or AT&TT/ different environment; $^{\#\#\#}$ p<0.001, difference from the same genotype, different environment.

alcohol use disorder that appeared as developmentally related to anxiety (**Paper III**). Involvement of the A-allele in trait anxiety and in obsessive-compulsive disorder was found also by Glotzbach-Schoon et al. (2013) and Lennertz et al. (2013), and is expected from animal studies (e.g., Pape et al. 2010). So, sleep-related problems in subjects with AA genotype that were already observable at age 15 most likely reflect affective dysregulation that could lead to psychiatric disorders diagnosed in young adulthood, as sleep-related problems have been found to be in positive correlation with depression symptoms (reviewed by Shochat et al. 2014). However, further analysis revealed that significant predictors for affective/anxiety disorders were sex, higher number of SLEs and *NPSR1* AA genotype in combination with adverse family environment; whereas bedtime, sleep duration and sleep-related problems recorded at age 15 were not associated with the disorder later in life (Table 3). This could mean that *NPSR1* independently influences both sleep-related measures and affective/anxiety-related dysregulation.

Table 3 Predicting affective/anxiety disorders in the older cohort of the ECPBHS, results from simultaneous logistic regression. Bold – significant effects.

Predictor	Wald statistic	*p*	Exp(β) [95% confidence interval]
Sex (females)	**9.01**	**0.003**	**2.76 [1.42–5.36]**
NPSR1 (**AA** vs T-allele)	0.44	0.507	1.40 [0.52–3.82]
Sleep-related problems	0.90	0.341	0.96 [0.88–1.05]
Bedtime	0.95	0.330	1.20 [0.83–1.74]
Sleep duration	0.43	0.513	0.79 [0.39–1.60]
SLE at age 15 (high)	**4.18**	**0.041**	**1.99 [1.03–3.86]**
Warmth in the family	0.62	0.431	0.75 [0.37–1.54]
***NPSR1* × Warmth (AA/low)**	**6.51**	**0.011**	**5.79 [1.50–22.3]**
Constant	9.67	0.002	

4.6. Closing remarks

In this dissertation, we have concentrated on neuropeptide S (NPS) receptor NPSR1 gene functional rs324981 A>T polymorphism ($Asn^{107}Ile$), that is relatively newly identified research target. We have demonstrated that *NPSR1* influences the development of personality, hyperactivity, anxiety, depressiveness, self-esteem, suicidality, affective/anxiety disorders, alcohol use and alcohol use disorders, and sleep-related measures.

Association of *NPSR1* with various physiological and psychological measures is not surprising as NPS and NPSR are localised in brain areas that

regulate basic physiological functions like arousal and circadian rhythms. In addition, NPS is known to modulate HPA axis function and the release of monoamines that could explain the sex, age and environment-dependent effects.

Our findings suggest that the *NPSR1* A/T polymorphism is associated with affective and anxiety disorders, suicidal behaviour, and alcohol use disorders in a sex- and environment-dependent manner. The genotype by sex/gender interactions were profound. In females, the lower *NPS*-ergic activity in A-allele carriers and especially in AA homozygotes bears a risk for affective and anxiety-related dysregulation already in adolescence. The risk for developing maladaptive traits is significantly higher in case of adverse family environment. As a consequence, females with the AA genotype had reported suicidal behaviour more frequently and had developed affective/anxiety disorders by age 25. Affective and anxiety related dysregulation may render them vulnerable also to alcohol use. Consequently, some females carrying the A-allele develop AUD already in young adulthood.

In males, an impulsivity-related early-onset pathway to AUD occurs in T-allele carriers and in particular in TT homozygotes: already in adolescence, they exhibit more ADHD symptoms and impulsivity that make them vulnerable to alcohol use, especially when experiencing adverse environment. As a consequence, many males carrying the T-allele develop AUD by young adulthood. In males, a delayed-onset pathway to AUD is also suggested for the AA genotype: the increase of adaptive impulsivity, extraversion and openness to experience by age 25 in males with AA genotype can make them vulnerable to higher alcohol use. However, future studies have to reveal whether AUD will develop on this basis in male AA subjects in later age.

NPSR is clearly a tempting target for drug development as it affects emotion regulation, arousal and alcohol abuse. E.g., nasal administration of NPS demonstrated by Lukas & Neumann (2012) in animals could lower anxiety and promote behavioural activation in humans in case of affective/anxiety disorders as well but possible side effects including panic attacks in some individuals elicited by higher autonomic arousal caused by higher NPSergic activity warrant more studies. So, although the results presented in this dissertation are based on population-representative samples, the *NPSR1* influence on various measures while accounting with environment should be tested on other ethnic groups, and in different types of societies as well. In addition, future directions should address the distinct pattern of emotion regulation by A- and T-allele carriers.

5. CONCLUSIONS

We have found that the functional *NPSR1* A/T polymorphism ($Asn^{107}Ile$) is associated with various measures in general population in age-, sex- and environment-dependent manner.

1. *NPSR1* genotype is associated with personality, but this association will become apparent mostly by young adulthood due to changes in scores from age 18 to 25. The TT genotype effects on personality could be interpreted as adverse, especially in women. The effects of the AA genotype on personality were advantageous, especially in the case of a favourable family environment, and more so for men. However, sensitivity of the AA genotype to family environment could lead to detrimental changes in personality, e.g., higher neuroticism and lower extraversion, in both sexes.
2. *NPSR1* T-allele carriers, especially male TT homozygotes, exhibited high levels of ADHD-related symptoms and impulsivity that were further accentuated by stressful life events. While *NPSR1* was associated with hyperactive/inattentive behaviour as rated by teachers already durig the childhood of the subjects, *NPSR1* related changes in self-reported impulsivity emerged more prominently in young adulthood.
3. Females with the *NPSR1* AA genotype who had reported adverse family environment in adolescence had higher scores of trait anxiety and depressiveness, and lower self-esteem. Male AA homozygotes had higher depressiveness only in case of adverse family relations.
4. The hypothesised high vulnerability of AA genotype to emotion dysregulation was corroborated by the highest prevalence of affective/ anxiety disorders by age 25 in female AA homozygotes who had experienced adverse family environment in adolescence.
5. Female AA homozygotes also reported suicidal behaviour more frequently, and again, this association was further accentuated by adverse family environment.
6. In females, both AUD and harmful alcohol use were more prevalent in A-allele carriers. In contrast, in males, AUD was more frequent in T-allele carriers, especially if exposed to adverse environments during adolescence. Alcohol use was higher in male T-allele carriers in adolescence as well. However, in adulthood, the risk allele for higher alcohol use for males was the A-allele as in females, suggesting distinct pathways to higher alcohol use by *NPSR1* in males.
7. The associations of *NPSR1* with AUD and alcohol use were mediated by personality and hyperactivity. This is in line with the hyperactivity/ impulsivity and personality regulating role of the *NPSR1* that differs in males and females. In females, the probable lower *NPS*-ergic activity in A-allele carriers and especially in AA homozygotes bears a risk for affective and anxiety-related dysregulation already in adolescence that also renders these subjects vulnerable to alcohol use. Consequently, some

females carrying the A-allele develop AUD already in young adulthood. In males, an impulsivity-related early-onset pathway to AUD occurs in T-allele carriers and in particular in TT homozygotes: already in adolescence, they exhibit more ADHD symptoms and impulsivity that make them vulnerable to alcohol use, especially when experiencing adverse environment. As a consequence, many males carrying the T-allele develop AUD by young adulthood. In males, a delayed-onset pathway to AUD is also suggested for the AA genotype: the increase of adaptive impulsivity, extraversion and openness to experience by age 25 in males with AA genotype can make them vulnerable to higher alcohol use. However, future studies have to reveal whether AUD will develop on this basis in male AA subjects in later age. Nevertheless, altogether these findings highlight the possibility that variants of a single gene can contribute to the development of a psychiatric disorder through several behavioural and neurobiological pathways.

8. The *NPSR1* A/T polymorphism is associated with sleep-related phenotypes. While the AA genotype was associated with higher frequency of sleep-related problems both at adolescence and adulthood, and later bedtime in adolescence, the TT genotype was associated with later bedtime in adulthood. Both AA and TT homozygous subjects slept less in adulthood if they had experienced adverse environment in adolescence. These associations of genotype and sleep could in part explain the psychiatric disorders associated with the *NPSR1* genotype.

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SUMMARY IN ESTONIAN

Neuropeptiid S ja vaimne tervis: NPS retseptori genotüübi ja keskkonna roll isiksuseomaduste ja psühhiaatriliste häirete kujunemisel

Hiljutiavastatud neuropeptiid S (NPS) avaldab närilistel omapärast mõju, suurendades aktiivsust ja virgust samaaegselt ärevuse alandamisega. Inimesel on NPS retseptori geenis *NPSR1* funktsionaalne A>T polümorfism (rs324981), mille T-alleeli kooditud retseptorvalk on signaali vahendamisel tõhusam. T-alleeli kandlus on seotud suurema virguse, kuid ka paanikahäirega, mistõttu on T-alleeli hakatud pidama riskialleeliks. Väitekirjas kajastatud rahvastikupõhiste uuringutega lõime tasakaalustatuma pildi *NPSR1* toimimisest, leides nii soost, vanusest kui keskkonnast sõltuvaid seoseid.

NPSR1 T-alleeliga isikud, eriti TT genotüübiga mehed, olid hüperaktiivsemad ja impulsiivsemad, ning stressirikkad elusündmused rõhutasid neid omadusi veelgi. Kui *NPSR1* mõjutab aktiivsus-tähelepanuhäirega seotud käitumist õpetajate hinnangute alusel juba lapseeas, siis genotüübi seosed eneseraporteeritud impulsiivsuse ja muude isiksuseomadustega avaldusid tugevamalt täiskasvanueas tänu muutustele 18-ndast 25-nda eluaastani. TT genotüübi mõju isiksusele võiks tõlgendada pigem ebasoodsana, ja seda eriti naiste jaoks. Seevastu AA genotüübiga uuritavatel, eriti meestel, oli soodsam isiksuseprofiil, ja seda eriti heade peresuhete korral. Samas reageerisid mõlemast soost AA genotüübiga isikud tugevalt kehvale perekeskkonnale neurootilisuse ja maladaptiivse impulsiivsuse kasvu ning ekstravertsuse ja adaptiivse impulsiivsuse alanemisega.

Leidsime, et naistel, kellel on madalaima aktiivsusega *NPSR1* genotüüp, AA, võib juba teismeeas meeleolu ja ärevuse regulatsiooniga raskusi olla. Mitteadaptiivsed jooned ilmnesid neil sagedamini just kehvade peresuhete korral ja väljendusid kõrgemates püsiärevuse ja depressiivsuse skoorides ning madalas enesehinnangus. AA genotüübi oletatav emotsionaalne haavatavus leidis veelgi kinnitust: kõige rohkem afektiivseid ja ärevushäireid esines 25ndaks eluaastaks neil AA genotüübiga naistel, kes olid kogenud teismelisena kehvi peresuhteid. AA genotüübiga naised raporteerisid 18-aastaselt ka kõige sagedamini enesetapukatseid, ja jällegi oli seos tugevam just kehvade peresuhete korral.

NPSR1 oli seotud ka alkoholi kuritarvitamise ja alkoholisõltuvusega. Naistel olid nii alkoholi kuritarvitamine kui sõltuvushäire sagedasemad A-alleeli kandjatel. Seevastu meestel esines oluliselt rohkem teismeea alkoholitarvitamist ning sõltuvushäireid T-alleeli kandjate hulgas, ja seda eriti teismelisena kogetud stressirikaste elusündmuste puhul. Ometi oli meestel täiskasvanuna sarnaselt naistega alkoholitarvitamise riskialleeliks A-alleel, mis viitab meestel *NPSR1*-st sõltuvatele erinevatele alkoholitarvitamise mustritele. Kuna *NPSR1* seoseid sõltuvushäirete ja alkoholi tarbimisega vahendasid isiksusejooned ja impulsiivsus, sobides kokku *NPSR1* sooti erineva rolliga hüperaktiivsuse/impulsiivsuse ja isiksuse kujundamisel, saab kirjeldada järgnevaid sõltuvuse

tekkimise radasid. A-alleeliga naistel, eriti AA homosügootidel, tähendab tõenäoliselt madalam *NPS*-egiline aktiivsus raskusi meeleolu ja ärevuse regulatsiooniga juba teismeeas, mis teeb nad alkoholi tarvitamisele vastuvõtlikuks. Seega kujunes osadel A-alleeliga naistest 25ndaks eluaastaks välja alkoholisõltuvushäire. Meestel eksisteerib impulsiivsusega seotud varajase algusega sõltuvuse tekkimise rada: *NPSR1* T-alleeliga mehed, eriti TT homosügoodid, olid hüperaktiivsemad ja impulsiivsemad, mis tegi nad vastuvõtlikuks alkoholi tarvitamisele, eriti stressirikkaid elusündmusi kogedes. Seetõttu pole üllatav, et T-alleeliga meeste hulgas oli oluliselt rohkem sõltuvushäireid. Nagu mainitud, raporteerisid huvitaval kombel sarnaselt naistega alkoholiga liialdamist täiskasvanuna hoopis AA genotüübiga mehed, mis viitab võimalikule täiendavale hilise algusega sõltuvuse tekkimise rajale: AA meestel tõusid adaptiivne impulsiivsus ja avatus oluliselt 25-ndaks eluaastaks, ning nad olid ka alkoholi kuritarvitamisele vastuvõtlikumad. Kas neil ka hiljem sõltuvus kujuneb, jääb tulevikus selgitada.

NPSR1 A/T polümorfism on seotud ka erinevate unefenotüüpidega. AA genotüübiga isikutel oli sagedamini uneprobleeme nii teismeeas kui täiskasvanuna, ja nad läksid teismelisena hiljem magama; seevastu TT genotüübiga isikud läksid täiskasvanuna hiljem magama. Nii AA kui TT homosügoodid magasid täiskasvanuna lühemat aega, kui nad olid teismelisena halvemat keskkonda kogenud. Genotüübi seosed uneprobleemidega sobivad *NPSR1* rolli selgitamiseks psühhiaatrilistes häiretes.

NPSR on huvipakkuv sihtmärk ravimiarenduseks, kuna mõjutab virgust, emotsionaalseid reaktsioone ja alkoholi kuritarvitamist. Analoogselt loomkatsetega võiks NPSi ka inimestel kasutada näiteks meeleolu- ja ärevushäirete puhul käitumusliku aktiviseerijana ja ärevuse alandajana. Samas aga tuleks uurida ja olla ettevaatlik võimalike kõrvaltoimete suhtes, kaasa arvatud võimalus, et NPS-i indutseeritud autonoomse närvisüsteemi aktivatsioonist tekivad mõnel inimesel paanikahood. Kuigi väitekirjas esitatud tulemused on saadud rahvastikupõhiseid valimeid kasutades, tuleks *NPSR1* mõju koostoimes keskkonnaga uurida ka teistel etnilistel gruppidel ja erinevates ühiskondades. Eraldi tähelepanu tuleks suunata AA ja TT homosügootide erinevate emotsioonide reguleerimise viiside sügavuti tundma õppimiseks.

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Publications:

1. **Laas K**, Reif A, Herterich S, Eensoo D, Lesch KP, Harro J (2010) The effect of a functional *NOS1* promoter polymorphism on impulsivity is moderated by platelet MAO activity. *Psychopharmacology* 209:255–261.
2. Mäestu J, Lätt E, Rääsk T, Sak K, **Laas K**, Jürimäe J, Jürimäe T (2013) Ace I/D polymorphism is associated with habitual physical activity in pubertal boys. *The Journal of Physiological Science* 63:427–434.
3. Kiive E, **Laas K,** Akkermann K, Comasco E, Oreland L, Veidebaum T, Harro J (2014) Mitigating aggressiveness through education? The mono-amine oxidase A genotype and mental health in general population. *Acta Neuropsychiatrica* 26:19–28.

4. **Laas K**, Reif A, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) A functional NPSR1 gene variant and environment shape personality and impulsive action: A longitudinal study. *Journal of Psychopharmacology* 28:227–236.
5. **Laas K**, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) Interaction of the neuropeptide S receptor gene $Asn^{107}Ile$ variant and environment: contribution to affective and anxiety disorders, and suicidal behaviour. *International Journal of Neuropsychopharmacology* 17:541–552.
6. **Laas K**, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) Neuropeptide S receptor gene variant and environment: Contribution to alcohol use disorders and alcohol consumption. *Addiction Biology (in press)*. doi:10.1111/adb.12149

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Teaduspublikatsioonide loetelu:

1. **Laas K**, Reif A, Herterich S, Eensoo D, Lesch KP, Harro J (2010) The effect of a functional *NOS1* promoter polymorphism on impulsivity is moderated by platelet MAO activity. *Psychopharmacology* 209:255–261.
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5. **Laas K**, Reif A, Akkermann K, Kiive E, Domschke K, Lesch KP, Veidebaum T, Harro J (2014) Interaction of the neuropeptide S receptor gene $Asn^{107}Ile$ variant and environment: contribution to affective and anxiety disorders, and suicidal behaviour. *International Journal of Neuropsychopharmacology* 17:541–552.
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