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What is This?
A functional NPSR1 gene variant and environment shape personality and impulsive action: A longitudinal study

Kariina Laas1, Andreas Reif2,4, Evelyn Kiive1, Katharina Domschke2, Klaus-Peter Lesch2,5, Toomas Veidebaum3 and Jaanus Harro1

Abstract
Neuropeptide S and its receptor NPSR1 are involved in the regulation of arousal, attention and anxiety. We examined whether the NPSR1 gene functional polymorphism Asn107Ile (rs324981, A>T) influences personality, impulsivity, and attention-deficit/hyperactivity disorder (ADHD)-related symptoms in a population-representative sample, and whether any eventual associations depend on age, sex, family relations and stressful life events (SLE). We used self-reports or teachers’ ratings for both the younger (n=593) and older (n=583) cohort of the longitudinal Estonian Children Personality, Behaviour and Health Study. Males with the TT genotype displayed more ADHD-related symptoms. Adaptive Impulsivity and Extraversion increased the most from age 18 to 25. While highest increases were observed in AA men, TT women exhibited the largest decreases. For participants with the AA genotype, Warmth in family was inversely associated with Neuroticism, and positively associated with Extraversion and Adaptive impulsivity. High exposure to SLE increased impulsivity and ADHD scores in TT genotype subjects. We conclude that the NPSR1 A/T polymorphism is associated with impulsivity, ADHD symptoms and personality, mirroring the activity- and anxiety-mediating role of NPSR1. Heterozygous individuals were the least sensitive to environmental factors, whereas subjects with the AA genotype and TT genotype reacted to different types of environmental adversities.

Keywords
Neuropeptide S, NPSR1, anxiety, arousal, ADHD, impulsivity, personality, stress

Introduction
Neuropeptide S (NPS) acts as a neuromodulator and influences arousal- and anxiety-related behaviour in rodents via its receptor NPSR1 (Reinscheid and Xu, 2005; Rizzi et al., 2008; Xu et al., 2004, 2007). NPSR1 is a G-protein coupled receptor first identified as an orphan receptor GPR154, with its gene located on chromosome 7p14.3. Among single-nucleotide polymorphisms (SNPs) in the NPSR1 gene, rs324981 A>T results in change of an amino acid (Asn107Ile). NPS has an approximately 10 times higher agonist potency at the T-allele-encoded NPSR1, leading to higher signal transduction efficiency (Reinscheid et al., 2005). Rodent studies prompted to test for an association of rs324981 with human diseases, especially anxiety disorders, and indeed the NPSR1 Asn107Ile polymorphism is associated with anxiety disorders and arousal. Okamura et al. (2007) and Domschke et al. (2010) found that the T-allele was overrepresented among patients with panic disorder. The T-allele was also related to anxiety sensitivity and heightened autonomic arousal, reflected by increased heart rate, during a behavioural avoidance test (Domschke et al., 2010). NPSR1 T-allele carriers also show increased amygdalar responsiveness to fear-relevant stimuli (Dannlowski et al., 2011) and increased fear appraisal together with increased rostral dorso-medial prefrontal cortex activity (Raczka et al., 2010), suggesting that NPSR1 contributes to anxiety and related disorders by regulating fear-related brain circuits. Pharmacological compounds that reduce anxiety, such as benzodiazepines, typically also reduce arousal and activity but the NPS system acts differently, as in animal studies central administration of NPS produces anxiolytic-like effects and arousal concurrently (Lukas and Neumann, 2012; Rizzi et al., 2008; Wegener et al., 2012; Xu et al., 2004). NPS has a direct effect on the hypothalamic–pituitary–adrenal axis at the level of the hypothalamus to elicit the increase of plasma adrenocorticotropic hormone and corticosterone (Smith et al., 2006). Thus the association of the gain-of-function T-allele with anxiety could arise from heightened arousal in T-allele carriers in response to environmental stimuli, but because in animal studies administration of NPS appears to be simultaneously activating and anxiolytic, it is not at all clear how increased arousal in T-allele carriers can result in pathological anxiety in some individuals.
**Table 1.** The number of subjects with complete data on genotype for NPSR1 Asn<sup>N107</sup>Ile (A/T) polymorphism and respective self-report measures by study wave and gender (males/females).

<table>
<thead>
<tr>
<th>Scale</th>
<th>Younger cohort</th>
<th>Older cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 y</td>
<td>15 y</td>
</tr>
<tr>
<td><strong>Personality measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Big Five-based self-reports&lt;sup&gt;1&lt;/sup&gt;</td>
<td>–</td>
<td>203/250</td>
</tr>
<tr>
<td>Impulsivity measures (self-reports)</td>
<td>–</td>
<td>213/254</td>
</tr>
<tr>
<td>ADHD-related scales (teachers’ ratings)</td>
<td>Hyperactivity scale</td>
<td>219/255</td>
</tr>
<tr>
<td></td>
<td>SNAP-IV</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>ASRS self-report</td>
<td>–</td>
</tr>
<tr>
<td><strong>Environmental factors (self-reports)</strong></td>
<td>Stressful life events (SLE high//low)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Tartu Family Relationship Scale&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(high//low)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98/106 FR</td>
</tr>
</tbody>
</table>

<sup>1</sup>Self-report instruments described in text.
<sup>2</sup>SLEs are divided into low and high SLE groups by median split (//).
<sup>3</sup>Family relations are divided into low and high Warmth (W), Maltreatment (M) and Family Relations (FR) groups by median split (//).

Attention-deficit/hyperactivity disorder (ADHD) is a clinically and genetically heterogeneous neurodevelopmental disorder characterized by inattention, heightened activity, impulsivity and emotional dysregulation as well as frequent co-morbid conditions such as anxiety disorders (Jacob et al., 2007). Thus NPS-ergic neurotransmission as a modulator of activity and arousal may have an influence on impulsivity and other ADHD-associated symptoms, and related anxiety-like behaviours. Okamura et al. (2007) did not find any association of rs324981 with ADHD in an underpowered German trio sample, but it remains unknown whether NPSR1 influences activity and impulsivity in the general population.

Affective and anxiety disorders may develop in consequence of an interplay between biological predisposition, such as genetic variation in gene expression, and environmental factors like adverse life events and family environment (e.g. Domshche and Reif, 2012; Harro, 2010). To date, there has been only one gene × environment (G×E) interaction study with NPSR1 probing the Anxiety Sensitivity Index (Klauke et al., 2012) which, however, was limited by the retrospective assessment of life events, the restrictions to severe life stress, and the examination of a rather restricted set of psychometric scales. Extending this topic, it was demonstrated in an animal study that stress induces release of NPS in the amygdala (Chauveau et al., 2012; Jüngling et al., 2008). Such evidence suggests that subjects with more effective NPS-ergic neurotransmission, for example subjects with the NPSR1 TT genotype, should be able to deal better with stress; that is, are more resilient. Nevertheless, as discussed above, so far the T-allele has rather been associated with psychiatric conditions.

In this study we examined whether the functional NPSR1 Asn<sup>N107</sup>Ile polymorphism influences personality, impulsivity and hyperactive/inattentive behaviours, and whether any eventual associations depend on age, sex, stressful life events (SLEs) and family relations.

**Methods and materials**

**The sample**

This study was carried out on the Estonian sample of the European Youth Heart Study (1998/99), which was subsequently incorporated into the longitudinal Estonian Children Personality Behaviour and Health Study (ECPBHS). The rationale and procedure of sample formation have been described elsewhere in detail (Harro et al., 2001; Tomson et al., 2011). The total number of subjects in the first wave 1998/99 was 1176; 583 in the younger cohort, the follow-ups were in 2001 (n=453, M<sub>Age</sub>=15.6±0.6). The follow-up studies for the younger cohort were in 2004 (n=483, M<sub>Age</sub>=15.3±0.5) and 2007 (n=453, M<sub>Age</sub>=18.3±0.5); for the older cohort, the follow-ups were in 2001 (n=453, including 62 additional subjects, M<sub>Age</sub>=18.4±0.7) and 2008 (n=540, M<sub>Age</sub>=24.7±0.7). The number of subjects with valid genotype and psychometric data is given for each analysis separately in Table 1. All the subjects were of Caucasian origin. The study was approved by the Tartu University Ethics Review Committee on Human Research.

**Measures**

**Personality.** Personality traits of the five-factor model were measured 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 (EBBF), which is a shorter and semantically simplified questionnaire (Harro et al., 2009; Laidra et al., 2006). Personality
ADHD symptoms were the alleles at the NPSR1 Asn<sup>107</sup>Ile polymorphism genotyping. The alleles at the NPSR1 rs324981 (Asn<sup>107</sup>Ile) SNP locus were amplified from DNA isolated from venous blood samples as previously described (Domschke et al., 2010). Genotype frequencies were in Hardy–Weinberg equilibrium. The NPSR1 A/T polymorphism was genotyped in 563 subjects in the younger and in 575 in the older cohort (Table 2). Genotype distribution in this sample representative of the Estonian population (AA 25.8%, AT 50.0% and TT 24.2%; both cohorts together) differs significantly from German samples (AA 29.9%, AT 48.6% and TT 21.5%, Domschke et al. (2010) and Klaue et al. (2012) combined) as well as from a Japanese sample (AA 21%, AT 43% and TT 36%; Okamura et al. 2007, panic disorder and schizophrenia cohort).

**Table 2. NPSR1 Asn<sup>107</sup>Ile genotype distribution in the ECPBHS sample.**

<table>
<thead>
<tr>
<th>NPSR1 genotype</th>
<th>Younger cohort</th>
<th>Older cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>males</td>
<td>females</td>
</tr>
<tr>
<td>AA</td>
<td>71 (13%)</td>
<td>78 (13%)</td>
</tr>
<tr>
<td>AT</td>
<td>129 (23%)</td>
<td>151 (27%)</td>
</tr>
<tr>
<td>TT</td>
<td>68 (12%)</td>
<td>66 (12%)</td>
</tr>
<tr>
<td>Total</td>
<td>268 (48%)</td>
<td>295 (52%)</td>
</tr>
</tbody>
</table>

**Data of the younger cohort were collected at ages 15 (EPIP-NEO) and 18 (NEO-PI-R), and of 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.**

**Impulsivity.** Self-reports for different facets of impulsivity were completed at ages 15 and 18 for the younger cohort and at ages 18 and 25 for the older cohort. We used the Adaptive and Maladaptive Impulsivity Scale (Eensoo et al., 2007; 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).

**Scales for ADHD-related symptoms.** ADHD symptoms were reported by teachers (described by Kiive et al., 2010) using the Hyperactivity Scale and SNAP-IV (Table 1). The older cohort was administered the ASRS scale at age 25. The Hyperactivity Scale consists of three subscales (Aggressiveness, Motor restlessness, and Concentration difficulties), and Hyperactivity score was calculated by summing the scores of Motor Restlessness and Concentration Difficulties (af Klinteberg and Oreland, 1995); SNAP-IV consists of two subscales, Inattention and Hyperactivity/impulsivity. All the scales were also dichotomized by upper 5% for the hypothetical presence of ADHD for examining categorical NPSR1 associations.

**Stressful life events and family environment.** History of SLEs was self-reported and subjects were divided into low and high SLE exposure groups by median split (Table 1). 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).

**Family relations.** Family relations were self-reported with the Tartu Family Relationships Scale that consists of four subscales, combined to higher-order scales Warmth (Closeness and Support) and Maltreatment (Neglect and Abuse) (Kiive et al., 2010; Paaver et al., 2008). Subjects were divided into low and high groups by median split. A single measure of family relationships was formed by subtracting scores of Maltreatment from Warmth, higher scores indicating generally more positive family relationship.

**Statistical analysis**

Subjects were analysed by NPSR1 genotype groups. Self-reported personality, impulsivity, and SNAP-IV and ASRS scores were transformed into z-scores after assessing overall age-dependent changes described in the next section. Mixed-effects analysis of variance, for example multilevel models (MLM), analysis of variance and covariance (AN(C)OVA) and correlation analysis were used for analysing effects of NPSR1 genotype, age, sex, SLE and family relations on personality, impulsivity and ADHD-related measures with SAS 9.1. Age and SLE as time-varying covariates were used as random factors in MLM models; a between–within method was used for degrees of freedom. Contrasts were calculated for significant model effects. Results from MLM are reported in the form of F-statistic, raw p-value and confidence intervals (CI) for group means; AN(C)OVA results are reported as F-statistic, raw p-value and CI for group means. Genotype distribution was performed by Fisher’s exact test.

**Results**

**Sex differences and age-dependent changes**

Scores of Neuroticism, Extraversion and Openness to experience decreased, and Agreeableness and Conscientiousness increased in the younger cohort as previously reported (Harro et al., 2009). This also applied to the older cohort from ages 15–18 and most domains at age 18–25, only Openness to Experience increased from age 18 to 25 (at age 18 M=104.4, SD=18.8 and at age 25 M=119.7, SD=18.3, p<0.001). In both cohorts, women scored higher on each personality dimension except Extraversion, for which no sex difference was found (Table 3). In both cohorts,
We found neither NPSR1 genotype main effect nor interaction effect with sex on personality measures, but age-dependent changes in Extraversion, Agreeableness and Openness to Experience were dependent on NPSR1 genotype (Table 3). A significant NPSR1 × Age effect revealed that the scores in Openness to Experience decreased in subjects with the TT genotype so that the difference between AA and TT genotypes reached significance at age 25. We also found an NPSR1 × Age × Sex effect on Extraversion: the heterozygotes did not change with age, but from age 18 to 25 the scores of Extraversion increased in AA genotype men and decreased in TT women. The NPSR1 × Age × Sex effect on Agreeableness in the older cohort demonstrated that this trait decreased from age 15 to 25 most in AA men and increased most in AT women. Thus, the NPSR1 genotype had some effects on personality development from age 15 to age 25.

The changes in Openness to experience and Extraversion, but not in Agreeableness, were explained by changes in impulsivity. On the other hand, adjustment for ADHD symptoms, SLE or family relations did not influence these results.

NPSR1 × environment interaction effects on personality. There was no significant NPSR1 × SLE effect on personality measures, but in both cohorts subjects with the AA genotype were most sensitive to family relations as expressed in scores of Neuroticism and Extraversion (Table 3). NPSR1 × Family relations × Age effect on Neuroticism revealed that favourable family environment lowered and adverse environment increased negative emotionality most in subjects with the AA genotype in both cohorts. Also, scores of Extraversion were influenced most in AA genotype by family environment in both cohorts; a similar effect on Openness to Experience was observed only in the older cohort. Maltreatment affected Agreeableness in subjects with the TT genotype. This was not found in younger cohort, but data on family relations were collected at a different age.

### Table 3. T-statistics of MLM for NPSR1, Sex, family environment (Family) and NPSR1 interaction effects on personality. Bold indicates a significant model.

<table>
<thead>
<tr>
<th>Personality</th>
<th>Sex</th>
<th>NPSR1</th>
<th>NPSR1 × Age</th>
<th>NPSR1 × Age × Sex</th>
<th>Family&lt;sup&gt;1&lt;/sup&gt;</th>
<th>NPSR1 × Family × Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OC</td>
<td>OC</td>
<td>OC</td>
<td>OC</td>
<td>OC</td>
<td>OC</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>4.94***</td>
<td>-0.33</td>
<td>-0.30</td>
<td>-0.07</td>
<td>W -4.92***</td>
<td>2.53*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M 7.78***</td>
<td>-0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FR -7.70***</td>
<td>2.19*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W 3.32**</td>
<td>-2.01*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M -2.15**</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FR 3.44***</td>
<td>-2.43*</td>
</tr>
<tr>
<td>Extraversion</td>
<td>1.51</td>
<td>-0.29</td>
<td>-1.63</td>
<td>-2.25*</td>
<td>W 0.86</td>
<td>-2.12*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M 0.86</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FR -0.05</td>
<td>-2.9*</td>
</tr>
<tr>
<td>Openness to Experience</td>
<td>7.22***</td>
<td>-1.34</td>
<td>-1.96*</td>
<td>-0.45</td>
<td>W 3.65***</td>
<td>-0.36</td>
</tr>
<tr>
<td>Agreeableness</td>
<td>5.10***</td>
<td>-0.59</td>
<td>0.47</td>
<td>3.05***</td>
<td>M -4.29**</td>
<td>-2.28*</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>2.25*</td>
<td>-0.23</td>
<td>1.07</td>
<td>1.58</td>
<td>FR 5.06***</td>
<td>0.63</td>
</tr>
</tbody>
</table>

<sup>1</sup>Family environment (Family) measures are Warmth (W), Maltreatment (M) and Family Relations (FR).

ADHD scores and Maladaptive impulsivity decreased and Adaptive impulsivity increased, and while men had higher scores of Adaptive impulsivity, women scored higher on Maladaptive impulsivity. Males also had higher scores of ADHD measures compared with females at all ages. SLEs correlated moderately with family environment (e.g. Spearman’s rho at age 18 SLE × Warmth -0.507, p<0.01; SLE × Maltreatment 0.527, p<0.01). Both warmer family environment and lower number of SLEs were linked to lower Neuroticism (SLE t=2.92, p=0.004; Warmth t=-4.92, p<0.001), Maladaptive impulsivity (SLE t=3.47, p<0.001; Warmth t=-3.23, p=0.001) and hyperactive/inattentive behaviour (e.g. SNAP-IV scores, SLE t=6.58, p<0.001; Warmth t=3.45, p<0.001), and also higher Extraversion (Warmth t=3.32, p<0.001), Agreeableness (SLE t=3.70, p<0.001; Warmth t=3.63, p<0.001) and Conscientiousness (SLE t=-2.23, p=0.026; Warmth t=4.39, p<0.001). All these reported values were derived from the older cohort, but similar significant patterns were observed in the younger cohort as well. Openness to Experience was influenced only by SLE (t=2.22, p=0.027), and not family relations.

**NPSR1 genotype effects on personality**

**NPSR1 interaction effects with Age and Sex on personality.** We found neither NPSR1 genotype main effect nor interaction effect with sex on personality measures, but age-dependent changes in Extraversion, Agreeableness and Openness to Experience were dependent on NPSR1 genotype (Table 3). A significant NPSR1 × Age effect revealed that the scores in Openness to Experience decreased in subjects with the TT genotype so that the difference between AA and TT genotypes reached significance at age 25. We also found an NPSR1 × Age × Sex effect on Extraversion: the heterozygotes did not change with age, but from age 18 to 25 the scores of Extraversion increased in AA genotype men and decreased in TT women. The NPSR1 × Age × Sex effect on Agreeableness in the older cohort demonstrated that this trait decreased from age 15 to 25 most in AA men and increased most in AT women. Thus, the NPSR1 genotype had some effects on personality development from age 15 to age 25.

The changes in Openness to experience and Extraversion, but not in Agreeableness, were explained by changes in impulsivity. On the other hand, adjustment for ADHD symptoms, SLE or family relations did not influence these results.

NPSR1 × environment interaction effects on personality. There was no significant NPSR1 × SLE effect on personality measures, but in both cohorts subjects with the AA genotype were most sensitive to family relations as expressed in scores of Neuroticism and Extraversion (Table 3). NPSR1 × Family relations × Age effect on Neuroticism revealed that favourable family environment lowered and adverse environment increased negative emotionality most in subjects with the AA genotype in both cohorts. Also, scores of Extraversion were influenced most in AA genotype by family environment in both cohorts; a similar effect on Openness to Experience was observed only in the older cohort. Maltreatment affected Agreeableness in subjects with the TT genotype. This was not found in younger cohort, but data on family relations were collected at a different age.

**NPSR1 genotype effects on impulsivity**

**NPSR1 main effect and interaction with sex and age on impulsivity.** NPSR1 main effect and interaction with Sex emerged only by age 25 (Table 4; Figure 1). From age 18 to 25, Adaptive impulsivity scores increased in subjects with the NPSR1 AA genotype and decreased in TT genotype carriers so that the difference between genotypes reached significance (Figure 1(a)) at the age of 25 years. The effect was driven by sex differences (Figure 1(b)). Family relations as covariates had almost no effect...
on the results: 1) \(NPSR1 \times \text{Age with Warmth as a covariate} \quad t(1, 245)=-2.53, \ p=0.012; \) 2) \(NPSR1 \times \text{Age with Warmth} \quad t(1, 245)=-3.80, \ p=0.0002. \) Thus, by age 25, adaptive impulsivity was the highest in AA males and the lowest in TT females. We found no significant \(NPSR1\) main effect or interactions with Sex or Age on Maladaptive impulsivity (Table 4).

\(NPSR1 \times \text{SLE interaction effects on impulsivity.}\) Higher number of SLEs increased Maladaptive impulsivity but not Adaptive impulsivity in both cohorts (Table 4). In both cohorts, subjects with the TT genotype had higher scores of impulsivity in the case of high SLE, mostly at age 18 (Table 4). In the younger cohort the \(NPSR1 \times \text{SLE effect on adaptive impulsivity was not yet detected at age 15 but was present by age 18. In the older cohort, scores of Disinhibition were higher at age 18 in subjects with the TT genotype who had experienced more SLE (Figure 2(a)), and the influence of SLE on TT genotype was still apparent at age 25. There was no \(NPSR1 \times \text{SLE effect on the other maladaptive type of impulsivity, Thoughtlessness, though the \(NPSR1 \times \text{SLE \times Age effect on overall Maladaptive impulsivity, like on Disinhibition, confirmed that subjects with TT genotype react to SLE most strongly (Table 4).}\)
NPSR1 × family relations effect on impulsivity. We found NPSR1 × Family relations effects on impulsivity in both cohorts (Table 4). Family relations affected more the subjects with the AA genotype compared with T-allele carriers. Subjects with the AA genotype had, in combination with favourable family environment, high adaptive and low maladaptive impulsivity, and in the case of adverse family environment, high maladaptive and low adaptive impulsivity (Figure 2(b), Table 4). This held for both measurement times in the older cohort (the effect even strengthened at age 25: NPSR1 × Family relations × Age) but only for age 15 in the younger cohort (Table 4). Thus, the family environment reported at age 15 only influenced impulsivity at the same age, but family environment reported at age 18 still had an effect at age 25.

NPSR1 × Family environment effects on impulsivity in both cohorts were driven by sex differences: in a favourable family environment the AA males had the highest scores of adaptive and lowest maladaptive impulsivity, whereas TT females had the lowest scores of adaptive impulsivity. Thus, in both cohorts AA males can take advantage of a favourable family environment, in terms of impulsivity.

NPSR1 genotype effects on ADHD-related scales

NPSR1 main effect and interaction with sex and age on ADHD-related scales. NPSR1 had effects on ADHD-related scales in both cohorts over all ages (Figure 3(a)–(c)); TT genotype males had significantly more ADHD-related symptoms than TT females; in addition, TT males had significantly higher scores compared with A-allele carriers on SNAP-IV/ASRS (Figure 3(a)) and Motor Restlessness (Figure 3(c)) scores. The NPSR1 × Sex effect on SNAP-IV/ASRS total scores in the older cohort at ages 18 and 25 was in part explained by exposure to the SLE ($p=0.088$ when SLE is a covariate). SLE as a covariate did not explain, however, the effect on Aggressiveness in the older cohort measured at ages 15 and 18, $t(1, 565)=2.22, p=0.027$. This suggests that in younger age the effects in both cohorts were independent of SLE. We separated the subjects with upper 5% scores of ADHD scales as a proxy measure for possible ADHD diagnosis and compared them with the remaining group, as the prevalence of ADHD is between 3–7% in school-age population (Cormier, 2008).
NPSR1 TT genotype was overrepresented among the highest 5% of SNAP-IV total scores in the younger cohort at age 15 (Fisher’s exact tests: for all the genotypes $p=0.03$; TT genotype vs. A-allele $p=0.016$, OR=3.08, CI(1.27–7.49)); the only two girls in the high-score group were T homozygotes (Table 5). At age 18 the effect on SNAP-IV scores disappeared, and no genotype effect on dichotomized SNAP-IV scores at age 18 and ASRS at age 25 was found in older cohort. However, from those 21 subjects who belonged to the top 5% of SNAP-IV scores at age 15, only five subjects were rated by teachers at age 18 and they still belonged to the upper 5% of scores (four of them had TT genotype). Thus dropout of the extremes could be one reason why we could not detect more significant results with extreme ADHD scores by the NPSR1 genotype.

NPSR1 × SLE interaction effects on ADHD-related scales. The interaction effect of NPSR1 and SLE on ADHD symptoms was found only in the younger cohort: both males and females with high number of SLEs and at least one T-allele exhibited higher Inattention ($t(1, 220)=2.46, p=0.014$), while the increase in Motor Restlessness was observed only in males (NPSR1 × SLE × Sex effect, $t(1, 170)=-2.85, p=0.0046$). In addition to the NPSR1 × SLE effect on Inattention, the whole SNAP-IV scale yielded a similar significant effect ($t(1, 220)=2.11, p=0.034$), data not shown. We found no significant NPSR1 × Family relations effect on ADHD scales.

Discussion

We report here that the NPSR1 Asn$^{107}$Ile polymorphism influences hyperactive and inattentive behaviour, personality and impulsivity independently, and also in interaction with sex, age and environmental factors. The key findings in our study were: 1) subjects with the NPSR1 TT genotype exhibited the highest level of impulsive action; 2) subjects with the TT genotype were sensitive to SLE; and 3) subjects with the AA genotype were sensitive to the family environment. A simplified overview of the results is presented in Figure 4. The data were derived from three measurement waves of two separate cohorts of a population-representative sample of Estonians: the younger cohort was observed at ages 9, 15 and 18, and the older cohort was observed at ages 15, 18 and 25. The effects on ADHD-related symptoms were apparent in both cohorts, although more evident in the younger cohort, whereas the NPSR1’s main effects and interaction with sex on personality and impulsivity were observed primarily in the older cohort due to differences at age 18 and even more in young adulthood at age 25. This is plausible, as some developmentally inappropriate 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 and impulsivity were observed in both cohorts again.

The NPSR1 Asn$^{107}$Ile polymorphism has recently been described as a modulator of anxiety and arousal, with evidence for the T-allele being overrepresented in subjects with higher arousal and anxiety (Dannlowski et al., 2011; Domschke et al., 2010; Klauke et al., 2012; Raczka et al. 2010). In our study, the NPSR1 effects on personality and impulsivity were most adaptive for AA males and less adaptive for TT females, supporting the previous findings about the female-dominant adverse effect of the T-allele. A more complex role of NPSR1 is, however, suggested by findings on ADHD-related behaviour, with TT genotype males exhibiting the highest number of observable symptoms, and the NPSR1 × environment interactions. The strongest effects in the study, which would also survive Bonferroni correction, were mainly

![Figure 4. Simplified overview of the results of the NPSR1 effects and interactions with SLE and family relations.](image-url)
related to \textit{NPSRI} effects on impulsivity. These effects were supported by significant but weaker effects of the \textit{NPSRI} on ADHD scores and personality.

\textbf{Impulsive action}

Changes in personality, mostly from age 18 to 25, were dependent on \textit{NPSRI} genotype. The rather opposing shifts for TT females support the previous findings in females with the T-allele conveying risk of anxiety- and arousal-related psychopathology. Impulsivity, but not the ADHD-related symptoms, SLE or family environment explained the genotype effects on Openness to Experience and Extraversion, indicating that impulsivity had a role in the development of personality in early adulthood.

The \textit{NPSRI} A/T polymorphism influenced impulsivity in both cohorts. Impulsivity is a heterogeneous construct (e.g. Congdon and Canli, 2005), understood as a tendency to act in an immediate and uncontrolled manner, characterized by rapid error-prone information processing. Impulsivity has, nevertheless, been proposed to be separable into two domains: functional and dysfunctional impulsivity (Dickman, 1990). The first, a rather adaptive impulsivity, refers to quick decision-making according to situation and gaining from rapid action, while dysfunctionally or maladaptively impulsive subjects can be described as acting with little forethought in a non-reflective manner in spite of negative consequences. The main effect of \textit{NPSRI} on impulsivity was found only in the older cohort due to changes in Adaptive impulsivity from age 18 to 25, so no effects could have been expected in the younger cohort. The \textit{NPSRI} effect was driven by sex differences: males with the AA genotype had the highest, and females with the TT genotype the lowest scores of Adaptive impulsivity by age 25. The similar effect on Extraversion disappeared when accounting for impulsivity, and so did the \textit{NPSRI} effect on Openness to Experience, showing the influence of impulsivity on personality.

Nevertheless, \textit{NPSRI} in interaction with environmental factors had effects on both adaptive and maladaptive types of impulsivity in both cohorts. Subjects with the TT genotype had significantly higher impulsivity in the case of high SLE, which may indicate an increase in activity in response to stress. This fits with the modest effects of \textit{NPSRI} on ADHD-related behaviour rated by teachers, where TT males had more symptoms, and SLE further increased the scores. Next, subjects with the AA genotype, and especially AA males, gained most from favourable family relations by reacting with elevated Adaptive impulsivity and with lowered Thoughtlessness, possibly because the supporting environment lowers anxiety. However, an unfavourable family environment increased Thoughtlessness and Neuroticism and lowered Extraversion in subjects with the AA genotype, which may indicate the harmful effects of anxiety. So the \textit{NPSRI} effects on both types of impulsivities may reflect changes in balance between activity/arousal and anxiety, supported by animal studies where NPS-ergic neurotransmission concurrently promotes arousal and lowers anxiety (Lucas and Neumann, 2012; Rizzi et al., 2008; Wegener et al., 2012; Xu et al., 2004).

In both cohorts, males with the TT genotype of \textit{NPSRI} had more ADHD symptoms as observed by teachers. SLEs further increased the ADHD scores in the 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 more results by upper 5% 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-year-old subjects from the younger cohort participated in the next wave, only 63% of the extremely high ADHD score subjects did so.

As the TT males expressed increased activity in our study, and the higher arousal of the gain-of-function T-allele carriers has been established previously (e.g. Domschke et al., 2010), supported by animal models (Reinscheid, 2008), \textit{NPSRI} appears as a strong ADHD candidate gene. This is also supported by the results of the impulsivity-mediating role of the \textit{NPSRI} in this study. In addition, both ADHD patients and above-average impulsive individuals are highly responsive to situations and easily aroused emotionally, which is in accordance with the reactivity of the T-allele to emotional stimuli (Dannlowski et al., 2011; Raczk et al., 2010). On the other hand, Okamura et al. (2007) reported no association of the \textit{NPSRI} A/T polymorphism with ADHD, and searches for ADHD candidate genes had not pointed towards \textit{NPSRI} or its location on chromosome 7p14.3 (e.g. Banaschewski et al., 2010; Lesch et al., 2008; Neale et al., 2008). Thus \textit{NPSRI} may contribute to ADHD-like behaviour dimensionally through impulsivity and arousal in the general population.

In response to centrally administered NPS, the increased locomotor activity is accompanied by wakefulness and deprived sleep in rodent models (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 cause these symptoms (Pavonen et al., 2009). NPS-ergic neurotransmission has also a direct effect on the hypothalamic–pituitary–adrenal axis, therefore eliciting the modulation of arousal and anxiety by regulating the release of corticotrophin-releasing factor (Smith et al., 2006). Thus the human \textit{NPSRI} polymorphism may reflect a balancing mechanism for fighting drowsiness and low cortical activity, and boosting arousal, to the largest extent in males with the TT genotype, which mimics the ADHD symptoms. In the general population this means that subjects with the \textit{NPSRI} TT genotype have a better functioning arousal/anxiety system, which in males expresses itself with higher impulsivity and activity, in females the activity is paradoxically disinhibited.

\textbf{Stressful life events and family relations}

We demonstrated in both cohorts that \textit{NPSRI} homozygotes responded to stress, AA to family environment, and TT mainly to SLEs. The fact that the AA genotype (the least active genotype) was reactive to stress may demonstrate the elevation of anxiety in case of adverse family environment, as expected from animal studies where \textit{NPSRI} knockout mice express increased anxiety-like behaviour (Duangdao et al., 2009) and administration of NPS acts like anxiolytic (Jüngling et al., 2008; Lukas and Neumann, 2012; Wegener et al., 2012; Xu et al., 2004). The only family environment effect that influenced the TT genotype was the reduction of Agreeableness by maltreatment, but this was found only in the older cohort. Otherwise, subjects with the TT and not AA genotype were sensitive to adverse life events: they had significantly higher scores of impulsivity, Inattention and Motor Restlessness (the latter for males only). The sensitivity to environment of the T-allele is in accordance with human studies, where all the presumed adverse arousal and anxiety-related effects were limited to T-allele carriers. The \textit{NPSRI} TT genotype subjects were also more sensitive to SLEs in Klaue et al. (2012), possibly because NPS
activates the hypothalamic–pituitary–adrenal axis (Smith et al., 2006; Zhao et al., 2012) and may enhance the stress response. However, this contradicts the translation from another animal study which suggested that in humans the T-allele carriers should have better functioning in response to stress compared with A-allele carriers, as NPSR1 injection to amygdala evoked anxiolytic effects and facilitated extinction of conditioned fear responses in rodents (Jüngling et al., 2008). So the reason why heterozygotes are so different in interacting with environment is not clear. Adverse family environment and adverse life events are different stressors, although they correlate moderately. In our sample, these factors had quite similar overall effects on personality reports, impulsivity and ADHD-related symptoms, therefore the different reactions of NPSR1 genotypes on those two stressors were surprising. It is possible that the key is the subjectivity of the evaluation: while life events were mostly reported as ‘yes/no’ in a mainly objective manner, the family environment assessment was based on mostly subjective quantitative evaluations. Whether objective or subjective indicators of environmental stress evaluations are used affects the outcome (Monroe, 2008), and self-reports of adverse life events may underestimate their occurrence. In this study such a bias could affect the results if underreporting were genotype dependent. This cannot be excluded and merits attention in future studies.

However, there were no differences in evaluating family environment by NPSR1. In addition, the NPSR1 A/T polymorphism influences emotional evaluations, and T-allele carriers react more strongly to fear-related emotional stimuli, possibly because of higher arousal in T-allele subjects (Dannlowski et al., 2011; Domschke et al., 2010; Raczka et al., 2010). Thus the reaction of subjects with the TT genotype to SLE could be related to arousal, and the reaction of the AA genotype to family environment must be anxiety-related, as derived from animal studies. Without knowing the exact neural mechanisms we conclude that NPSR1 A and T homozygotes perceive family environment and adverse life events in a different way, resulting in distinct outcomes in personality, impulsivity and ADHD symptoms.

In summary, NPSR1 influences hyperactive/inattentive behaviour rated by teachers already in childhood, whereas NPSR1 related changes in self-reported personality and impulsivity emerge more strongly in young adulthood. NPSR1 TT genotype effects on personality and impulsivity were adverse especially for women; teacher-rated ADHD symptoms were more abundant for TT males. The effects of the NPSR1 AA genotype on personality and impulsivity were advantageous, especially in the case of a favourable family environment, and more so for men. Nevertheless, the sensitivity to family environment of the subjects with the AA genotype led to detrimental changes in scores of Neuroticism among other scales in the case of adverse family relations, which may reflect aspects of anxiety in accordance with animal studies. In contrast, a different type of environmental factor, SLE, influenced both males and females with the NPSR1 TT genotype. Differently from homozygotes, subjects with the NPSR1 AT genotype had relatively stable scores across the time, and their reactions to SLEs and adverse family environment were modest, so heterozygosity of NPSR1 seems to increase resilience. The pattern of the effects where TT males had most of the ADHD symptoms and reacted to SLE with increased impulsivity leads us to a conclusion that the NPSR1 TT genotype may reflect heightened arousal also in humans, as expected from animal studies.

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Conflict of interest
The authors declare that there are no conflict of interest.

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