Lonely Reflections
Variations in Adolescents’ Trait and State Loneliness

Eeske van Roekel
Lonely Reflections
Variations in Adolescents’ Trait and State Loneliness

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
en ter verkrijging van de graad van doctor in de psychologie
aan de KU Leuven op gezag van rector prof. dr. R. Torfs

in het openbaar te verdedigen
op vrijdag 16 mei 2014 om 13.30 uur precies

by

Geesje Henrika (Eeske) van Roekel
geboren op 24 januari 1985
te Ede
## Table of Contents

### Chapter 1
- General Introduction

### Part I: Daily Life Processes

### Chapter 2
- Loneliness in the daily lives of adolescents: An Experience Sampling Study examining the effects of social contexts

### Chapter 3
- Trait and state levels of loneliness: Examining developmental and cultural differences in adolescence

### Chapter 4
- Loneliness, affect, and adolescents' appraisals of company: An Experience Sampling Method (ESM) study

### Chapter 5
- Loneliness in the daily lives of late adolescents: Testing a socio-cognitive model

### Chapter 6
- The negative company we keep: High levels of negative social experiences in early adolescents’ daily lives

### Part II: Genetic Influences

### Chapter 7
- Loneliness in adolescence: Gene-environment interactions involving the serotonin transporter gene

### Chapter 8
- The dopamine D2 receptor gene, perceived parental support, and adolescent loneliness: Longitudinal evidence for gene-environment interactions

### Chapter 9
- Oxytocin receptor gene (OXTR) in relation to loneliness in adolescence: Interactions with sex, parental support, and DRD2 and 5-HTTLPR genotypes

### Chapter 10
- The oxytocin receptor gene (OXTR) in relation to state levels of loneliness in adolescence: Evidence for micro-level gene-environment interactions

### Appendix I
- S-HTTLPR and DRD2 genotypes in relation to state loneliness in adolescence

### Chapter 11
- General Discussion

### References

### Dutch Summary (Nederlandse Samenvatting)

### Publications

### Acknowledgements (Dankwoord)

### Curriculum Vitae

---

**Promotoren**
- Prof. dr. R.C.M.E. Engels
- Prof. dr. L. Goossens (KU Leuven, België)

**Copromotoren**
- Dr. M. Verhagen
- Prof. dr. R.H.J. Scholte

**Manuscriptcommissie**
- Prof. dr. A. Cillessen
- Prof. dr. S. Branje (Universiteit Utrecht)
- Dr. P. Kuppens (KU Leuven, België)
- Prof. dr. A.J. Oldehinkel (Rijksuniversiteit Groningen)
- Dr. M. Wichers (Universiteit Maastricht)
Chapter 1
General Introduction
Human beings are characterized by the need to belong (Baumeister & Leary, 1995), a fundamental need to bond with others. This need to belong motivates people to form and maintain interpersonal relationships. When the quantity or quality of these interpersonal relationships is not sufficient, people can experience loneliness (Baumeister & Leary, 1995). Loneliness is defined as the negative emotional response to a perceived discrepancy between the desired and actual quality or quantity of one’s social relationships (Perlman & Peplau, 1981).

Transient feelings of loneliness are likely to be experienced by everyone from time to time. Experiencing these transient feelings of loneliness does not necessarily have negative consequences, as these feelings may motivate people to actively seek social contact (e.g., Gardner, Pickett, Jefferis, & Knowles, 2005). In contrast, chronic feelings of loneliness seem to have severe mental and physical health consequences, such as anxiety (Lasgaard, Goossens, Bramsen, Trillingsgaard, & Eiklid, 2011), depression (Cacioppo, Hughes, Waite, Hawkley, & Thisted, 2006; Vanhalst, Klimstra, et al., 2012), higher risk for cardiovascular disease (Casi, Harrington, Moffitt, Milne, & Poulton, 2006), less salubrious sleep (e.g., Hawkley, Preacher, & Cacioppo, 2010; Kurina et al., 2011), and poorer immune responses (Pressman et al., 2005). Importantly, higher levels of loneliness are found to increase chances of mortality by as much as 50% (Holt-Lunstad, Smith, & Layton, 2010). Given these negative consequences, it is important to examine the factors that can predict and maintain feelings of loneliness.

In this general introduction, we will first provide an introduction to the concept of loneliness, discuss the importance of examining loneliness in adolescence, and describe the different theoretical perspectives on loneliness. Second, we will elaborate on what is known about the daily experiences of lonely individuals, and how loneliness is experienced in daily life. Third, we will discuss the genetic and environmental factors that play a role in trait and state levels of loneliness.

**Loneliness Defined**

**What is Loneliness?**

In the present thesis, we used the definition of loneliness as presented by Perlman and Peplau (1981), in which loneliness is defined as the negative emotional response to a perceived discrepancy between the desired and actual quality or quantity of one’s social relationships (Perlman & Peplau, 1981). This definition comprises both an affective and a cognitive component. The cognitive component is that individuals have to perceive a discrepancy between their actual and desired social relationships. This is important, as it implies that loneliness is not just a synonym for objective social isolation, but merely reflects perceived social isolation. The affective component of the definition is that the perceived discrepancy has to lead to negative emotions. In other words, the experience of
this discrepancy should be unpleasant. In addition, the definition differentiates between the quantity and quality of social relationships, which indicates that people can feel lonely because they have fewer friends compared to others, or they can have the same number of friends, but feel dissatisfied with the quality of their relationships with these friends.

**Loneliness in Adolescence**

Adolescence is an important period in life in which many physical, emotional, and social changes occur. In the social domain, peer relationships become more complex, and are characterized by higher levels of intimacy and loyalty, compared to childhood (Steinberg & Morris, 2001). Adolescents become more involved in small peer groups (i.e., cliques), but also identify themselves with larger groups (i.e., crowds), a tendency that emerges in early adolescence (Brown & Klute, 2006; Davey, Yucel, & Allen, 2008), and they also become interested in romantic relationships (Collins, Welsh, & Furman, 2009). In line with these developments, adolescents are particularly sensitive to peer rejection, compared to children and adults (Kloep, 1999; Larson & Richards, 1994; O’Brien & Bierman, 1988), and this sensitivity seems to increase during adolescence (Silk et al., 2012).

Adolescence is also characterized by two important transitional phases: the transition from primary school to secondary school in early adolescence and the transition from secondary school to college in late adolescence. These transitions have a great impact on the social lives of adolescents, as they have to establish new social relationships, while maintaining existing relationships. In addition to this, the transition to college is often accompanied by leaving the parents’ home and moving to a new city. The heightened complexity of adolescents’ social worlds, combined with higher expectations of their peer relations, increased concern about their social status, and entering a new social environment (i.e., secondary school or college) could explain why feelings of loneliness are expected to be particularly present in adolescence (Heinrich & Gullone, 2006; Stroebe, van Vlet, Hewstone, & Willis, 2002).

However, surprisingly little research has examined the development of loneliness during adolescence longitudinally. A study on children showed that different loneliness trajectories could be distinguished (Jobe-Shields, Cohen, & Parra, 2011). The largest group of children showed stable low loneliness from age 9 to 11. A small group decreased in loneliness (12%), whereas a relatively large group increased in loneliness (23%) from childhood to pre-adolescence. In late adolescence, the general trend was a decrease in loneliness from age 15 to 20 (Vanhalst, Goossens, Luyckx, Scholte, & Engels, 2012). These studies show the development of loneliness in pre- and late adolescence only, but it would be expected that loneliness is particularly present in early adolescence as well, because of the transition to high school. Only one study has examined the development of loneliness longitudinally from childhood (age 7) to late adolescence (age 17), and indeed found evidence for a peak of loneliness in early adolescence, at age 13 (Qualter, Brown, et al., 2013). As this is the only study examining loneliness in early adolescence, further research on this topic is necessary. Hence, we examined the development of loneliness throughout adolescence (i.e., from age 13 to age 17, Chapter 7). In addition, we examined relationships in both early and late adolescent samples that recently went through important social transitions, as loneliness may be particularly present at these ages.

**Theoretical Perspectives on Loneliness**

As the need to belong is such a fundamental motivation for humans, many theorists have tried to explain what the antecedents, maintaining factors, and consequences of loneliness are. In the next section, we will focus on two theoretical perspectives, the evolutionary perspective and the (socio-)cognitive perspective, as the research in this dissertation builds on those theoretical frameworks.

**Evolutionary theory.** The evolutionary perspective hypothesizes that the experience of social pain (i.e., loneliness) is likely to be functional and adaptive (Cacioppo, Hawkley, et al., 2006). When a person experiences social pain, this may serve as a signal that something is wrong, which in turn functions as a motivation to go out and initiate or restore social relationships. From an evolutionary point of view, it is hypothesized that people who experienced social pain in response to social isolation were more likely to survive and pass on their genes than people who did not experience social pain, as the likelihood of survival is greater in a social community in which food is shared and people are protected from outside threats through stable social relationships. Hence, experiencing loneliness may increase the likelihood for survival and passing on one’s genes to the next generation.

Evidence for the evolutionary perspective comes, amongst other sources, from research examining responses to social rejection. Several studies have found that social exclusion activates the same brain areas (e.g., the anterior cingulate cortex) that are implicated in the affective responses to physical pain (for reviews, see N. I. Eisenberger & Cole, 2012; Naomi I. Eisenberger, 2013). Similarly to experiencing physical pain, this may indicate that experiencing social pain signals humans that something is wrong and actions have to be undertaken to avoid further harm.

**Socio-cognitive model.** Cacioppo and Hawkley (2009) have developed a socio-cognitive model that can explain how feelings of loneliness are sustained. According to this socio-cognitive model of loneliness, lonely people are characterized by (a) hypersensitivity to social threat and (b) hyposensitivity to social reward (Cacioppo & Hawkley, 2009). These two characteristics can result in a vicious circle in which lonely adolescents perceive their environment as more negative (i.e., hypersensitivity to social threat) and less positive (i.e., hyposensitivity to social reward), which can trigger (negative) behavioral confirmation processes, which in turn can lead to more negative interactions and negative affect, and finally result in even higher levels of loneliness. Hence, as lonely people perceive their social relationships as more negative, they eventually behave in such a way that their negative biases are actually reinforced by their social environment.
Several studies have found support for the two characteristics of the model. Regarding hypersensitivity to social threat, studies found that lonely people have greater visual attention for negative social stimuli than for negative non-social stimuli (Cacioppo, Norris, Decety, Monteleone, & Nusbaum, 2009) and view their daily activities as more threatening than non-lonely people (Hawkley, Burleson, Berntson, & Cacioppo, 2003). In addition, research in children (aged between 8-12 years) has shown that children with extreme levels of loneliness showed increased hostility to ambiguous situations of social exclusion, higher rejection sensitivity, and disengagement difficulties when viewing socially rejecting stimuli, which are all indicative of hypersensitivity to social threat (Quilter, Rotenberg, et al., 2013).

Regarding hyposensitivity to social reward, it has been found that the activation in reward areas in the brain (i.e., ventral striatum) in response to pleasant social pictures was lower in lonely compared to non-lonely people (Cacioppo et al., 2009), indicating that lonely people were not rewarded by pleasant social stimuli to the extent that non-lonely people were. On the other hand, a daily diary study in adults showed that lonely individuals benefitted more from positive social encounters than non-lonely people, in that their levels of negative affect decreased more when they experienced more positive social events (Russell, Bergeman, & Scott, 2012). This finding is in contrast with the socio-cognitive model, as it shows that lonely people are more rewarded by positive social encounters than non-lonely people.

In the present dissertation, both the evolutionary perspective and the socio-cognitive model are used as a basis for our research questions. For example, we examined the two characteristics of the socio-cognitive model in a sample of early adolescents (Chapter 4) and a sample of late adolescents (Chapter 5). In line with the evolutionary perspective, we examined whether certain genetic variants are related to loneliness in early adolescents (Chapter 7 – 10).

Part I: Daily Life Processes

Social Contexts

Adolescence is characterized by a shift in interpersonal relations. In early adolescence, levels of conflict with parents increase and adolescents report lower levels of closeness to their parents (Laursen, Coy, & Collins, 1998; Steinberg & Morris, 2001). In line with this trend, adolescents, compared to children, are found to spend less time with family (Larson & Richards, 1991) and experience less positive emotions in situations with family (Larson, 1983). Although these findings may indicate that parent-child relations become more negative during adolescence, these changes are merely a by-product of adolescents trying to gain autonomy (Collins, Laursen, Mortensen, Luebker, & Ferreira, 1997). Parents remain important for adolescents’ development, as they influence adolescents’ lives directly by monitoring their social relationships and activities, and indirectly by influencing their offspring’s social behaviors, attachment styles, and values (Steinberg & Morris, 2001).

Next to parents, peers become a very important social context in adolescence. The influence of peers can be very positive, as close, high quality friendships with peers are related to better adjustment in adolescence (e.g., Brown & Klute, 2006). On the other hand, peers can also have very negative effects on adjustment; as research has shown that peer rejection in adolescence is related to higher levels of externalizing and internalizing problems in later life (for review, see Deater-Deckard, 2001). Hence, being with peers with whom adolescents have close or intimate relationships may reflect a positive situation, whereas being with peers with whom adolescents do not have close or intimate relationships may have negative effects on adolescents.

Another change in social contexts in adolescence is that adolescents spend an increasing amount of time alone, compared to children (Larson & Richards, 1991). This time alone may be used constructively, that is, to concentrate on homework, for relaxing, reflecting on the self, or coping with emotions (e.g., Larson, 1997; Larson & Csikszentmihalyi, 1980; Larson, Csikszentmihalyi, & Graft, 1982), but is also found to be related to less positive and more negative emotions (Larson, 1990). Importantly, Larson (1997) found that a moderate time spend alone (i.e., 20% - 35% of waking hours) was related to higher wellbeing, indicating that adolescents do benefit from solitude, but only when they are alone for a limited amount of time.

Although this research highlights the importance of different social contexts in adolescence, very little is known about how adolescents experience and are affected by different social contexts when they are actually in them. A recent theory, developed by Beckes and Coan (2011), may shed light on how being in different social contexts affects individuals. According to Social Baseline Theory, being with others can be considered as a baseline state of relative calmness (i.e., social baseline), in which very little emotion regulatory efforts are needed. In this view, human brains are adapted to be in the proximity of other human beings who can protect the individual from outside threats (e.g., physical threats). Further, being part of a social network also makes it possible to share resources and goals, and care for each other (i.e., load sharing). Being with others hence requires the least emotional control and can help regulate emotions. On the other hand, being alone requires much more emotion regulation efforts, and heightened vigilance for potential threats, because there are no other people to share the risks with. In addition to this basic distinction between being alone and being with others, Social Baseline Theory (SBT) further differentiates between being with intimate versus non-intimate company. Here the assumption is that being with intimate company is the most positive situation to be in, as being with non-intimate company does not necessarily include the possibility for load sharing or lower vigilance for threats (as those non-intimate others may even represent potential threats to the individual). This line of reasoning was confirmed by an fMRI study investigating neural responses to threat in different social contexts (Coan, Schaefer, &
Davidson, 2006). People showed lowest responses to social threat when a high-quality relational partner was present, higher responses when they were in company of a stranger, and highest responses when they were alone.

Combining the implications from SBT with previous research on social contexts highlights the importance of examining how different real-life contexts affect adolescents. In the present dissertation, we examined how adolescents experience different social contexts in terms of positive and negative affect and loneliness in real life, by using momentary assessments (e.g., Chapter 2-5).

**State Loneliness**

Loneliness is typically examined as a trait (Marcoen, Goossens, & Caes, 1987; Russell, Peplau, & Cutrona, 1980) by means of questionnaires, that measure the extent to which individuals experience loneliness in general. Although traits are typically relatively stable, research on trait loneliness has shown that loneliness levels can fluctuate within individuals (Quater, Brown, et al., 2013; Vanhalst, Goossens, et al., 2012), and can be manipulated by using hypnosis (Cacioppo et al., 2000). These findings indicate that trait loneliness can also be transient. Further, loneliness may also fluctuate in daily life, depending on, among other things, the context people are in (Larson, 1981). Little is known about these daily manifestations of loneliness (i.e., state loneliness) and how they relate to trait levels of loneliness.

Only two studies have examined state levels of loneliness in adolescents. These studies have examined levels of state loneliness in different locations (i.e., home, school, and public places) and in different social contexts (i.e., situations alone and situations with others). The results of these studies showed that adolescents experienced the highest levels of state loneliness when they were alone (Larson, 1990), and adolescents were more lonely at home, compared to school and public places (Larson, 1981). Further, it was found that adolescents showed more extreme levels of state loneliness when they were alone on Friday and Saturday nights, indicating that timing of assessment was also important. Although these studies provide important information about state levels of loneliness, they are relatively dated and were conducted in US samples only. Hence, we aimed to replicate these results in a Dutch sample of early adolescents (Chapter 2), by examining differences in state levels of loneliness across different locations and different social contexts.

Although research has shown that adolescents often choose to be alone and use solitude constructively, as was mentioned earlier, the studies on state loneliness show that adolescents experience the highest levels of state loneliness when they are alone (Larson, 1981, 1990). From this point of view, solitude may be viewed as a stressor that increases feelings of loneliness. However, the studies examining the effects of solitude were limited in that they have only looked at concurrent associations (e.g., how lonely are adolescents when they are alone). We do not know whether being alone has a lasting effect on loneliness and whether entering the company of others can have a buffering effect.

Therefore, the lagged relations between social contexts and state loneliness were examined in Chapter 2.

**Characteristics of Trait Lonely People**

As mentioned earlier on, several studies have found that trait lonely people view their daily activities as more threatening and experience more negative affect and stress than non-lonely people (e.g., Hawkley et al., 2003). There may be several mechanisms that explain these heightened negative experiences in lonely people (Cacioppo, Hawkley, & Berntson, 2003). First, the differential exposure hypothesis states that lonely people experience lowered mood because they are exposed to more stressful and negative situations than non-lonely people. When this would be the case, it could indicate that lonely people do not necessarily respond more negatively to stressors, but that they have heightened stress levels because they indeed experience more negative situations than non-lonely people. On the other hand, the differential reactivity hypothesis states that lonely people may be exposed to similar numbers of negative situations, but that they respond to those negative situations more negatively than non-lonely people, with more intense negative emotions. Previous research has found little support for the differential exposure hypothesis, as lonely people were found to have similar numbers of reported major life stressors and life events as non-lonely people (Cacioppo et al., 2003). In addition, results from an Experience Sampling study showed that lonely adolescents did not differ from non-lonely people in the number of daily hassles or uplifts they reported, and that they spent a similar amount of time alone (Hawkley et al., 2003). However, support has been found for the differential reactivity hypothesis, in that lonely people view their daily activities as more threatening (Hawkley et al., 2003), perceive their interaction quality as more negative (Duck, Pond, & Leatham, 1994), and reported more severe hassles (Cacioppo et al., 2000), than non-lonely people do.

These studies indicate that lonely people indeed respond more negatively to different social stressors, but no studies have examined whether trait lonely adolescents experience higher levels of state loneliness when confronted with different social situations, which would be in line with the differential reactivity hypothesis. Hence, we do not know how and when high and low lonely adolescents experience state levels of loneliness in their daily lives, and whether lonely adolescents respond differently to specific social contexts than non-lonely adolescents. This is important to investigate, as knowledge on when lonely adolescents experience higher levels of state loneliness can provide starting points for intervention. In the present dissertation, the differential reactivity hypothesis was tested by investigating whether trait lonely adolescents experience different levels of state loneliness in response to social contexts, compared to non-lonely people (Chapter 3).

The two characteristics of the socio-cognitive model of loneliness, that is, hypersensitivity to social threat and hyposensitivity to social reward, are in line with the differential
reactivity hypothesis, as both characteristics imply that lonely people respond differently to social situations. As was discussed earlier in this Introduction, many studies provide evidence for the two characteristics. However, Russell and colleagues (2012) highlight the importance of examining these characteristics in daily life, as they found opposite effects on hypersensitivity for social reward in a daily diary study, compared to what was found in fMRI studies. In addition, most studies have only examined the extent to which lonely people perceived their environment as more negative and less positive, but not whether lonely people indeed responded more negatively and less positively to their own perceptions of the environment.

In the present dissertation, we examined the two characteristics of the socio-cognitive model in an innovative way, by measuring the real-life emotional responses of high and low lonely adolescents to negative perceptions of company (i.e., hypersensitivity to social threat) and positive perceptions of company (i.e., hypersensitivity to social reward) (Chapters 4 and 5). In this way, we investigated how adolescents responded to threatening and rewarding situations, which provided important insights into the subjective, real-life experiences of adolescents.

To some extent, negative perceptions of company may be normative, as everyone experiences judging or threatening company from time to time. However, as adolescence is characterized by an increase in negative events (e.g., conflicts with parents and peers, suspension from school, romantic relationship break-up) and adolescents are found to respond more negatively to those events compared to pre-adolescents (Larson & Ham, 1993), there may be social situations that are particularly negative for adolescents. Therefore, a further aim of the present dissertation was to examine within-person extreme levels of negative company (i.e., peaks in negative company) in Chapter 6. In this chapter, we further examined whether individual predictors (e.g., trait levels of loneliness) and situational predictors (e.g., time of day or type of company) were related to experiencing peaks in negative company. Additionally, we examined how adolescents responded to peaks in negative company, by examining levels of positive and negative affect during peak moments.

Part II: Genetic Influences

Based on the evolutionary theory of loneliness, it may be expected that loneliness has a genetic component, because people who experienced feelings of loneliness in response to social isolation would be more likely to survive and pass on their genes to the next generation. In this section, we will first discuss the behavioral genetic studies on trait loneliness and subsequently the molecular genetic studies that provide insights in which specific genes may be related to loneliness. It should be noted that we do not aim to provide a comprehensive overview of all molecular genetic studies that have been conducted, but merely report the findings that are relevant for our research questions.

Behavioral Genetic Studies

In behavioral genetics studies, similarities and differences in behavioral phenotypes are examined between people with different levels of genetic and environmental similarity, such as monozygotic and dizygotic twins (e.g., greater similarities in behavior between monzygotic twins compared to dizygotic twins would indicate that genetics play a role). Behavioral genetic studies on trait loneliness have found that trait loneliness is moderately heritable, with heritability estimates ranging from 45% to 55% in children (Barrels, Cacioppo, Hudzisak, & Boomsma, 2008; McGuire & Clifford, 2000), 75% in adolescents (Waaktaar & Torgersen, 2012), and 48% in adults (Boomsma, Willemsen, Dolan, Hawkley, & Cacioppo, 2005). These behavioral genetic studies indicate that loneliness has a significant genetic component. However, very few studies have further examined which specific genes are implicated in loneliness.

Candidate Gene Studies

Only one study has examined relations between a candidate gene (in the oxytocergic system) and trait loneliness (Lucht et al., 2009). Hence, further research is necessary to find out which genes may play a role in loneliness. Based on the two characteristics of the socio-cognitive model of loneliness, that is, hypersensitivity to social threat and hypersensitivity to social reward, we decided to examine candidate genes that play a role in three different neurotransmitter systems: the serotonergic system (5-HTT, serotonin transporter gene), the dopaminergic system (DA, dopamine receptor D2 gene), and the oxytocergic system (OT, oxytocin receptor gene).

First, the serotonin transporter (5-HTTPLR) genotype, a 44-bp insertion/deletion polymorphism located in the promoter region of the serotonin transporter gene, encodes for two allelic variants; the short allele and the long allele. Carrying one or two short alleles of this gene has been found to be related to negative outcomes, such as depression (for meta-analysis, see Clarke, Flint, Attwood, & Munafo, 2010). In addition, neuropsychological studies have shown that short allele carriers show higher amygdala activation in response to threatening stimuli (for meta-analysis, see Munafo, Brown, & Hariri, 2008) and have reduced connectivity between the amygdala and the perigenual anterior cingulate cortex (Pezawas et al., 2005), which is conceptualized as the feedback circuit that is involved in the extinction of negative affect. Hence, short allele carriers do not show overactivation of the amygdala in response to threat, but they also have problems with downregulating this overactivation. As the socio-cognitive model states that lonely people are characterized by hypersensitivity to social threat, it can be expected that this genotype is related to loneliness.

Second, the dopamine D2 receptor gene (DRD2 Taq1A C>T polymorphism consists of two allelic variants, the A1 allele and the A2 allele. Previous research has indicated that carrying at least one A1 allele is associated with reduced dopamine D2 receptor binding in brain reward areas (i.e., the ventral striatum; Thompson et al., 1997), which is hypothesized...
to lead to lowered reward experience (Blum et al., 1996). This link is further confirmed by neuropsychological studies, that showed that A1 carriers had less activation in brain reward areas when presented with a reward, compared to A2A2 genotypes (Cohen, Young, Baek, Kessler, & Ranganath, 2005). Because the socio-cognitive model of loneliness states that lonely people are hyposensitive to social reward, and an IMRI study has shown that lonely people show lowered reward responses in the same reward areas in the brain as A1 carriers, it may be expected that the DRD2 genotype is related to trait levels of loneliness.

Third, the oxytocin receptor gene (OXTR) rs53576 Single Nucleotide Polymorphism (SNP) encodes for two allelic variants: the A allele and the G allele. Although little is known about the functionality of this gene, that is, no studies have examined relations between the allelic variants and brain oxytocin levels or receptor functionality, the rs53576 variant has been implicated in several social behavioral phenotypes. For example, studies showed the A allele to be related to less sensitive parenting (Bakermans-Kranenburg & van IJzendoorn, 2008), less empathy and higher levels of stress (Rodrigues, Saslow, Garcia, John, & Keltner, 2009), and less sociality (Tost et al., 2010). In addition, A carriers displayed less nonverbal affiliative cues in social interaction and were rated as less prosocial than GG carriers (Kogan et al., 2011). Combining these findings, it would seem that the A allele is related to less positive social outcomes, which could include feelings of loneliness. This was indeed found in the only candidate gene study examining feelings of loneliness, which found that adults carrying the AA genotype of the rs53576 variant of this gene had higher levels of loneliness than individuals carrying a G allele (Lucht et al., 2009).

In the present dissertation, relations were examined between these three genotypes and trait loneliness by using a longitudinal design. The main advantage of this approach is that this allows us to take a developmental perspective, by looking at the development of loneliness throughout adolescence, instead of one-time assessments. Relations between the 5-HTTLPR genotype and the onset and development of trait loneliness were examined in Chapter 7, relations between the DRD2 genotype and trait loneliness were examined in Chapter 8, and in Chapter 9, we examined relations between the OXTR genotype and trait loneliness, including gene-gene interactions between the OXTR genotype and the DRD2 and 5-HTTLPR genotypes.

Although the candidate gene approach has provided some important results, there are some problems with this line of research. Meta-analyses and reviews on the effects of genes on behavioral phenotypes have indicated that many gene effects are not replicated. There may be several reasons for this lack of replication (Finan, Tennen, Thoemmes, Zautra, & Davis, 2012): (a) low phenotype reliability, (b) low power due to small effects and small samples, and (c) poor phenotypic specification. To some extent, these problems can be overcome by using the Experience Sampling Method. Regarding low phenotypic reliability, Experience Sampling studies increase the reliability of measures as the assessments take place in real-time, in natural environments. In this way, the ecological validity of the assessments is high, and recall bias is reduced as participants fill out the questions about that specific moment. In addition, because repeated measurements are used, the power of analysis increases (for simulation of power increases by multiple measurements, see Finan et al., 2012). Finally, to overcome poor phenotypic specification, several researchers have suggested that future studies should focus on examining intermediate phenotypes (or so-called endophenotypes) that decrease the distance between the gene and the phenotypic outcome by examining processes in between (Finan et al., 2012, Gottesman & Gould, 2003). Hence, in the present dissertation we examined micro-level effects of genes, by investigating relations between genes and state levels of loneliness. Although these micro-level effects may not exactly represent an intermediate phenotype, state levels of loneliness are more likely to be functional in terms of evolutionary benefits, and therefore state loneliness may represent a good phenotype for examining genetic effects. Up to now, no studies have examined relations between candidate genes and state levels of loneliness. Therefore, in addition to studying relations between genotypes and trait loneliness in Chapters 7-9, we examined micro-level relations between the OXTR gene and state levels of loneliness in Chapter 10. Further, we examined relations between DRD2 and 5-HTTLPR and state levels of loneliness (Appendix I).

Gene-Environment Interactions

Although candidate gene studies may provide interesting results and can indicate which genes play a role in loneliness, genes do not operate in isolation from the environment and may interact with environmental factors in predicting behavioral outcomes (Moffitt, Caspi, & Rutter, 2005; Rutter, 2007). In this way, certain genetic effects may only come to expression in particular environmental circumstances, and certain environmental predictors may only have an effect on people with certain genetic variants.

Until recently, the focus of gene-environment interaction research has been on examining ‘risk’ alleles that make individuals more vulnerable to negative environments. This line of research is based on the diathesis-stress model, which states that dual risks, that is, carrying a ‘risk’ allele and having experienced negative environmental stressors, lead to the most negative outcomes (e.g., Costello et al., 2002; Shanahan & Hofer, 2005). On the other hand, the differential susceptibility theory states that some individuals may not only be more negatively affected by negative environments, but also more positively affected by positive environments (Belsky & Pluess, 2009). Hence, certain genotypes may make individuals more susceptible to the environment in general. Even more recently, Pluess and Belsky (2013) have proposed the concept of vantage sensitivity, which refers to individuals who are more susceptible to positive environments exclusively. In order to be able to examine which of the three theories applies to specific gene-environment interaction results, it is necessary to include environmental variables on a continuum from negative to positive, which is different from a continuum from negative to an absence of negativity, which is often used in GxE research. In the present dissertation, we used environmental factors that concur with this notion.
Previous research on gene-environment interactions. Regarding the 5-HTTLPR gene, many gene-environment interaction studies on different phenotypes have been conducted. Meta-analyses showed that results are mixed. One meta-analysis indicated that all GxE studies on depression were underpowered and hence findings were compatible to chance findings (Munafo, Durrant, Lewis, & Flint, 2009), whereas another meta-analysis did find evidence for gene by stress interactions in depression (Karg, Burmeister, Shedden, & Sen, 2011). Yet another meta-analysis on children and adolescents specifically showed that short allele carriers in the total sample (i.e., including all different age and ethnicity groups) were more affected by negative environments, and that Caucasian children and adolescents also benefited more from positive environments (van IJzendoorn, Belsky, & Bakermans-Kranenburg, 2012). These findings combined indicate that short allele carriers are more affected by negative environments, and in some cases, also positive environments.

For the DRD2 genotype, results are mixed. In an overview of studies that found support for differential susceptibility, Belsky and Pluess (2009) showed that in four studies, A1 allele carriers were found to be more susceptible to their environment (Berman & Noble, 1997; Keltikangas-Jarvinen et al., 2007; Mills-Koonce et al., 2007; Propper et al., 2008), whereas one study found that A2 carriers were more negatively affected by stress (Elovainio et al., 2007). Based on these findings, A1 allele carriers are considered to be more affected by their environment.

Only a few studies have examined gene-environment interactions with the OXTR gene. Again, the results are inconsistent as to which genotype is more affected by the environment. Some studies found that GG genotypes were more affected by negative environments, compared to A allele carriers (Bradley et al., 2011; Sturge-Apple, Cicchetti, Davies, & Suot, 2012). Another study found that G carriers (i.e., AG and GG genotypes) showed lower stress responses after receiving emotional support, compared to A carriers (Chen, Kumsta, et al., 2011). Finally, A carriers were also found to be most affected by negative environments, in that they responded more negatively to stressful life events than GG genotypes (Poulin & Holman, 2013). These findings show that there is more support that GG carriers are more susceptible to their environment, but findings are mixed.

Importantly, no studies have examined gene-environment interactions in relation to loneliness. Therefore, one of the goals of the present dissertation was to examine interactions between the genotypes mentioned earlier and environmental predictors in relation to trait loneliness in Chapters 7 (5-HTTLPR genotype), 8 (DRD2 genotype), 9 (OXTR genotype), and in relation to state loneliness in Chapter 10 (OXTR genotype) and in Appendix I (5-HTTLPR and DRD2 genotype).

Previous research on environmental factors. As no gene-environment interaction studies on loneliness have been carried out, the choice for the environmental factors had to be based on research examining environmental predictors for loneliness in adolescence.
CHAPTER 1

General Issues

Before we move to the aims of the present dissertation, there are some general issues in Part I and II of the present dissertation that have to be mentioned.

Sex Differences

In adolescence, sex differences emerge in the prevalence of psychological disorders, in that girls tend to experience higher levels of internalizing problems, such as depression, than boys (see Hankin & Abramson, 2001 for review on sex differences in depression). For trait loneliness, results are less consistent. Some studies found girls to have higher levels of loneliness (Vanhalst, Klimstra, et al., 2012), others found that boys experienced higher levels of loneliness (Hoza, Bukowski, & Beery, 2000), but most studies found no sex differences in trait levels of loneliness (Bowler & Spencer, 2010; Jobe-Shields et al., 2011) (see also L. J. Koenig & Abrams, 1999 for a review on sex differences in loneliness).

Although these studies provide no definite answer to the question whether there are sex differences in loneliness, several studies on peer relations have shown that boys and girls do experience social relationships differently (for review, see Rose & Rudolph, 2006). For example, girls in general report receiving more provisions from their friendships, such as closeness, affection, nurturance, and acceptance (Jobe-Shields et al., 2011). These sex differences highlight the importance of considering possible differences between boys and girls in the present dissertation. Therefore, we controlled for sex in our analyses in all chapters, and further examined whether specific relations differed between boys and girls in some chapters (e.g., Chapter 1 and 5).

Regarding our genetic studies in Part II, previous research has indicated that genetic effects may be different for males and females. For example, studies on depression in adolescents reported sex differences in gene-environment interactions with the 5-HTTLPR genotype (Eley et al., 2004; Sjöberg et al., 2006) and several studies with the OXTR genotype also reported sex differences (Kogan et al., 2011; Lucht et al., 2009; Tost et al., 2010). The latter fact is not surprising, as research has shown that oxytocin receptors are partly upregulated by estrogen (e.g., Bale & Dorsa, 1995; M. Feng et al., 2009; Quiñones-Jenab et al., 1995), a sex hormone that is particularly present in females. Therefore, we examined gene by sex interactions in Chapters 7, 8, 9, and 10, and Appendix I, and controlled for sex in all analyses.

Specificity of Effects

Loneliness is highly correlated with depression (Cacioppo, Hughes, et al., 2006), which often raises the question whether loneliness is a separate construct, or merely a symptom of depression. Importantly, several studies have examined the interrelations between loneliness and depression. An evaluation of questionnaire items representing loneliness and depression has shown that loneliness items had very low loadings on the depression factor, and conversely that the depression items had very low loadings on the loneliness factor. This study therefore showed that loneliness and depression are separate constructs (Cacioppo, Hawkley, et al., 2006). Further, research has shown that loneliness and depression have different consequences for mental and physical health. For example, loneliness was uniquely related to blood pressure (Hawkley, Masi, Berry, & Cacioppo, 2006), and predicted mortality (Holt-Lunstad et al., 2010), whereas this was not the case for depression.

Several studies have tried to disentangle the causal relations between depression and loneliness. Research in both older adults and adolescents has found reciprocal relations between loneliness and depression, that is, loneliness predicted future depressive symptoms, and vice versa (Cacioppo, Hughes, et al., 2006; Vanhalst, Klimstra, et al., 2012). These findings show that loneliness and depression seem to be separate constructs, but these findings do not rule out the possibility that both constructs cause and maintain similar daily characteristics.

Regarding the two characteristics of the socio-cognitive model that we examine in Part I, previous research has shown that hypersensitivity to social threat and hypo-sensitivity to social reward also play a role in depression (Davey et al., 2008). Further, regarding the genetic studies in Part II, many studies have examined the genetic background of depression, and have found effects that may be similar in loneliness as well. Therefore, we controlled for depression in most chapters, so that we were sure that our findings were specific for loneliness, and not due to high correlations with depression.

The Present Dissertation

Overall Goal

The overall aim of the present thesis was to gain more insight into both the daily life characteristics of lonely adolescents and the genetic underpinnings of trait and state feelings of loneliness. In the first part, we filled the previously mentioned gaps in the literature by examining in two studies how state levels of loneliness develop across different locations and social contexts, and how trait and state loneliness are related to one another. In addition, we tested two characteristics of the socio-cognitive model of loneliness, that is, hypersensitivity to social threat and hypo-sensitivity to social reward, in both early and late adolescents. With regard to hypersensitivity to social threat, we further examined how adolescents responded to within-person extreme levels of negative company (i.e., peaks in negative company). In the second part, we extended previous behavioral genetic findings by examining several candidate genes in relation to trait and state loneliness, and whether these candidate genes interacted with social support in predicting loneliness by using both a longitudinal design as well as the ESM.
Study Design, Methodology and Sample Characteristics

Experience Sampling Method. In the present dissertation, we used the Experience Sampling Method to get insight in the daily lives of adolescents. In the ESM, individuals fill out multiple assessments per day, usually on random time points. Compared to more traditional, cross-sectional methods, this method has several important advantages, (a) it reduces recall bias as adolescents do not have to rate their feelings retrospectively, (b) it has high ecological validity, as adolescents fill out the questionnaires while they are living their lives, and (c) the reliability of the measures is high, as each variable is measured multiple times (Myin-Germeys et al., 2009).

Datasets. Several datasets were used in the present thesis (see Table 1). First, we used ESM data collected in early adolescents in 2010-2011. In this study among 303 early adolescents, a baseline questionnaire was administered in which demographic characteristics and trait loneliness were measured, followed by the sampling period 2 to 10 weeks later. The sampling period consisted of 6 days, on which adolescents received a smartphone that emitted signals at 9 random time points per day and each time they had to fill out a questionnaire on the smartphone. During the visit to administer the baseline questionnaires, saliva was collected for genotyping (see Chapters 2, 3, 4, 6, and 10).

Second, ESM data from Dutch late adolescents were used, that were collected in 2012. In this study, 223 first and second year college students were recruited. They first filled out a baseline questionnaire and, one week later, they participated in the ESM part. The sampling period lasted for 11 days, with five random signals per day. The signals were sent by means of emails, in which a link to an online questionnaire was provided (see Chapters 3 and 5).

Third, we used ESM data from the group of John Cacioppo, University of Chicago, US, that was collected in 1999. The sample consisted of 135 undergraduate students, who were selected so that low, middle, and high tertiles of trait loneliness were equally represented. One day prior to the ESM part, adolescents filled out a baseline questionnaire. The sampling period consisted of 7 days, with 9 random signals per day. Adolescents carried a wristwatch that emitted signals, after which they had to fill out paper-and-pencil versions of the questionnaire (see Chapter 3).

Fourth and finally, we used the ‘Family and Health’ data, a longitudinal study spanning five annual waves. Data were collected from 2002 to 2007, among 428 families, consisting of a father, mother, and two adolescent children. Only data from the youngest adolescents were used, because they were entering adolescence at the first wave (T1), making it possible to examine the development of loneliness throughout adolescence. Genotyping occurred at T4. As not all adolescents gave their consent for genotyping and due to drop out at wave T4, our final sample consisted of 307 adolescents.

### Table 1 Characteristics of the Different Datasets Used in the Present Dissertation

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Design</th>
<th>Data collection</th>
<th>Sample</th>
<th>Chapters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swinging Moods</td>
<td>Experience Sampling</td>
<td>Baseline assessment. 6 day sampling period, 9 random beeps per day</td>
<td>N = 303 adolescents, aged 13-16</td>
<td>2, 3, 4, 6, 10</td>
</tr>
<tr>
<td>ESM late adolescents</td>
<td>Experience Sampling</td>
<td>Baseline assessment. 11 day sampling period, 5 random beeps per day</td>
<td>N = 223 adolescents, aged 18-28</td>
<td>3, 5</td>
</tr>
<tr>
<td>ESM late adolescents US</td>
<td>Experience Sampling</td>
<td>Baseline assessment. 7 day sampling period, 9 random beeps per day</td>
<td>N = 135 adolescents, aged 18-24</td>
<td>3</td>
</tr>
<tr>
<td>Family and Health</td>
<td>Longitudinal</td>
<td>Five annual waves.</td>
<td>N = 307 adolescents, aged 13 at T1</td>
<td>7, 8, 9</td>
</tr>
</tbody>
</table>

Specific Goals of the Empirical Chapters

**Part I: Daily life processes.** Chapter 2 represents a study that examined state levels of loneliness in early adolescence, with a focus on different locations (i.e., home, school, and public places) and different social contexts (i.e., alone, family, friends, classmates, and others). In addition, we examined the temporal dynamics of social contexts on state levels of loneliness. As the effects of social contexts may differ for boys and girls, sex differences were tested as well.

The main goal of the study presented in Chapter 3 was to examine relations between trait and state levels of loneliness, and to test the differential exposure and differential reactivity hypothesis. Differences and similarities in these relations were examined across three different samples: (a) early adolescents from the Netherlands, (b) late adolescents from the Netherlands, and (c) late adolescents from the US.

In Chapter 4, the main aim was to test two characteristics of the socio-cognitive model of loneliness, that is, hypersensitivity to social threat and hyposensitivity to social reward, in early adolescents. We specifically examined whether trait lonely adolescents would be more negatively affected by negative perceptions of company, and less positively affected by positive perceptions of company.

The main aim of Chapter 5 was to examine and replicate the findings of Chapter 4 in a sample of late adolescents. Hence, we examined relations between perceptions of company and affect, and tested whether trait loneliness moderated these relations.

The study in Chapter 6 was set up to examine more extreme negative social situations in early adolescents. Specifically, we examined (a) whether individual characteristics (i.e., sex and loneliness) and situational characteristics (i.e., type of day, time of day, and type of company) were related to peaks in negative company and (b) how adolescents responded to peaks in terms of positive and negative affect.
Part II: Genetic influences. In Chapter 7, the main aim was to examine relations between 5-HTTLPR, parental support, and the onset and development of trait loneliness, including gene-environment interactions.

The study in Chapter 8 aimed to examine relations between the DRD2 genotype, parental support, and the onset and development of trait loneliness in adolescence. In addition, gene-environment interactions between the DRD2 genotype and parental support were examined.

The main aim of Chapter 9 was to investigate relations between the OXTR genotype and the intercept and slope of trait loneliness. In addition, gene-environment interactions with parental support, and gene-gene interactions between the OXTR genotype and the DRD2 and 5-HTTLPR genotype were tested.

In Chapter 10, we examined micro-level effects of the OXTR gene on state levels of loneliness. In addition, gene-environment interactions were tested between the OXTR gene and positive and negative perceptions of company. Finally, these relations were also examined for the 5-HTTLPR and DRD2 genotype in Appendix I.
Chapter 2

Loneliness in the daily lives of adolescents: An Experience Sampling Study examining the effects of social contexts

Resubmitted as:
CHAPTER 2

LONELINESS IN THE DAILY LIVES OF ADOLESCENTS

Introduction

Loneliness is defined as the negative emotional response to an experienced discrepancy between the desired and actual quality or quantity of one's relationships (Perlman & Peplau, 1981), and has been found to be particularly present in adolescence (e.g., Quilter et al., 2013). This increased prevalence may be explained by the fact that adolescence is an important period in life in which many physical, emotional, and social changes occur (Steinberg & Morris, 2001). In the social domain, peer relationships become more complex in adolescence, and are characterized by higher levels of intimacy and loyalty, compared to childhood (e.g., Steinberg & Morris, 2001). Adolescents get more involved in small peer groups (i.e., cliques), but also identify themselves with larger groups (i.e., crowds), a tendency that emerges in early adolescence (Davey et al., 2008). In addition, adolescents become interested in romantic relations (Collins et al., 2009). This heightened complexity of adolescents' social worlds, combined with higher expectancies of their peer relations and increased concern about their social status, could explain why loneliness is particularly present in adolescence (Heinrich & Gullone, 2006; Quilter, Brown, et al., 2013).

Loneliness is typically examined as a trait by using questionnaires (e.g., the Louvain Loneliness Scale for Children and Adolescents, Marcoen & Goossens, 1993; or the UCLA Loneliness Scale, Russell et al., 1978), measuring to what extent participants feel lonely in general. However, loneliness may not be a stable trait, but can fluctuate in daily life, depending on, amongst others, the context people are in (Larson, 1981). The main disadvantage of research on trait loneliness is that it does not take fluctuations and situational effects into account and therefore is not a good reflection of how loneliness is experienced in daily life. By measuring state levels of loneliness in real life, we can gain more knowledge about possible momentary fluctuations in state loneliness, as well as when and in which company adolescents experience state loneliness. In the present study, we focused on loneliness as a state rather than a trait, by examining momentary feelings of loneliness in the daily lives of adolescents.

Social Contexts

The time spent in different contexts changes in adolescence. Compared to pre-adolescents, adolescents are found to spend more time alone, less time with their family, and more time with friends (Larson & Richards, 1991). Based on research that showed that adolescents experience more conflicts with friends and family than children (Laursen, 1993), the increased importance of peer relations in adolescence (Steinberg & Morris, 2001), it is to be expected that the experience of this time in different social contexts also changes in adolescence. Very few studies have examined how adolescents experience social contexts when they are actually in it. Based on Social Baseline Theory (Beckes & Coan, 2011), differences between social contexts are to be expected. According to this theory, being with other individuals is considered as a baseline state of relative calmness,
as from an evolutionary point of view, being with other human beings would protect an individual from threats from outside the social group, and provides the opportunity to care for each other and share resources. On the other hand, being alone requires increased vigilance for threats because there is no one around to share the risk with, and increased emotion regulation efforts, as there are no others to help the individual to regulate his or her emotions. In this way, an individual has less to worry about when in a social environment, and hence this time in company of others would be experienced more positively than time alone.

However, as we humans further developed, and became part of more complex social groups, these benefits of being with others may not be applicable to every type of company. Therefore, SBT makes a further distinction between being with intimate versus non-intimate company (Beckes & Coan, 2011). Where intimate company can provide the above mentioned advantages, non-intimate company may not necessarily provide the opportunity to share outside risks or resources. In some circumstances, non-intimate company may even represent a potential threat to themselves, when they compete for similar resources for example, or reject an individual from their social group. In line with this, an fMRI study found that individuals showed the highest neural responses to potential threats when they were alone, lower responses when they were with a stranger, and lowest responses when they were with an intimate other (Coan et al., 2006). These findings highlight the importance of examining how adolescents experience different social contexts, when they are actually in them. Hence, we examined the extent to which adolescents experienced state loneliness in different social contexts in the present study.

**State Loneliness in Different Contexts**

A few studies have examined how adolescents feel in different social contexts and different locations. In line with SBT, a study by Schnieders and colleagues (2007) in Dutch adolescents (7th grade) showed that adolescents reported higher levels of positive affect and lower levels of depressed mood when they were in company, compared to being alone (Schnieders et al., 2007). Similar findings resulted from a study in an US sample (Mage = 12.6 years); Adolescents experienced lower levels of negative affect in situations with company, compared to situations in which they were alone (Silk et al., 2011). When examining the type of company adolescents were with, Silk et al. (2011) found that adolescents experienced a greater positive/negative affect ratio when they were with peers, compared to family members. This is in line with a study by Larson (1983), who found that adolescents (Grades 9 to 12) experienced more positive affect when they were with friends, compared to when they were with family. When considering the different locations, adolescents felt more anxious and irritated when they were at school compared to home and other places (i.e., public places and homes of friends), but experienced lower levels of depressed mood at school and other places, compared to home (Schnieders et al., 2007). In addition, they experienced the highest levels of positive affect in other places, compared to home and school.

Up to now, very few studies have examined state levels of loneliness in adolescence, and only two, relatively dated studies in predominantly white American samples (Grades 5 to 8) did so in different contexts (Larson, 1981, 1990). These studies examined whether state levels of loneliness were dependent on location (e.g., home versus school versus public places) (Larson, 1981) or on the social context (e.g., alone versus in company) (Larson, 1990). Results showed that adolescents had the highest levels of state loneliness when they were alone (Larson, 1990). When considering the location, adolescents were more lonely at home, compared to school and public places (Larson, 1981). However, these studies did not examine whether state levels of loneliness differed according to type of company (e.g., family, friends, or classmates), even though SBT highlights the importance of examining differences between intimate company versus non-intimate company.

In the present study, we examined differences in state loneliness between different contexts. We were not only interested in differences in state loneliness due to location, but also in differences between the types of company the adolescents were with, which has not been examined in previous studies. In addition, the previous studies examining state loneliness measured this construct with a single item (i.e., I feel lonely), whereas we measured state loneliness by a composite scale of four items (i.e., I feel lonely, isolated, left out, and abandoned). In this way, we obtained a more comprehensive measure of state loneliness.

**Temporal Dynamics of Social Contexts on State Loneliness**

As mentioned before, previous studies indicated that adolescents experience the highest levels of loneliness when they are alone (Larson, 1981, 1990). However, these studies merely examined concurrent associations. Although the findings from these studies indicate that adolescents experience being alone negatively, we do not know whether this negative experience has a lasting effect on adolescents and whether entering the company of others can have a buffering effect on the possible negative effects of being alone. A promising way to look at associations between being alone (i.e., solitude) and loneliness is by examining time-lagged effects (Marco & SulS, 1993). Marco and SulS (1993) proposed several ways in which a minor stressor (i.e., solitude) and emotions (i.e., loneliness) can be related. We examined whether these descriptive associations were applicable to the relationship between solitude (i.e., being alone), which was the minor stressor in our study, and state loneliness. We did this by comparing state loneliness between different social situations at T and T-1, in which Situation A refers to two consecutive moments of solitude, whereas in Situation B adolescents were alone at T, and in company at T-1. Situation C refers to moments in which adolescents were alone at T-1 and in company at T, and Situation D represents two consecutive moments of being in company.
The first descriptive association Marco and Suls (1993) discuss are spillover effects, which are applicable to situations in which solitude at time T-1 is related to higher levels of loneliness at the next assessment (T). This association would indicate that being alone has a lasting effect that is still present at the next time point. This would be the case when state levels of loneliness are higher in Situation A (two consecutive moments of solitude) compared to Situation B (no prior solitude) and when loneliness is higher in Situation C (prior solitude) compared to Situation D (no solitude).

Second, contrast effects occur when loneliness increases or decreases in response to the presence or absence of current solitude, being dependent on whether solitude was present or absent during the previous assessment. For example, contrast effects occur when adolescents are more lonely in situations when they were in the company of others at time T-1 and alone at time T (Situation B), compared to situations in which they were alone at both time points (Situation A). Finding a contrast effect in these situations indicates that entering solitude causes higher levels of loneliness than being alone at two consecutive time points. Another example of contrast effects is when loneliness is lower in situations when adolescents were alone and subsequently enter the company of others (Situation C), compared to situations in which adolescents were with company at two consecutive occasions (Situation D). A contrast effect in these situations may reflect a feeling of relief; adolescents feel less lonely when they enter the company of others, because they are relieved that they are not alone anymore.

Third, habituation occurs when two consecutive moments of solitude are not related to higher levels of loneliness at T than one episode of solitude (i.e., when state loneliness is equal in Situation A and Situation B). No research has been conducted on the temporal dynamics between the social context and state loneliness. However, Larson and colleagues (Larson, 1997; 1982) did examine how adolescents felt after a period of solitude. They examined differences in loneliness between Situations C and D, and found a contrast effect. After being alone and returning to the company of others (Situation C), adolescents experienced higher levels of positive affect than at times they were in the company of others, without having been alone before (Situation D).

In the present study, we examined differences in state loneliness between the previously described situations (Situation A versus Situation B and Situation C versus D), in order to decide which of the descriptive associations just mentioned are applicable (i.e., spillover, contrast, or habituation). In addition, because we expect differences between the type of company adolescents are in, we also took this into account. For example, we compared Situation A (consecutive moments of solitude) with different types of company in Situation B (with family, friends, or classmates at T-1, alone at T; see Table 1). Importantly, the results from these temporal relations will provide further insight into the experience of solitude in adolescence, and whether different types of social company can buffer possible negative effects of being alone.

### Table 1: Temporal Dynamics of Social Contexts on State Levels of Loneliness

<table>
<thead>
<tr>
<th>Model</th>
<th>Situation A</th>
<th>T-1</th>
<th>T</th>
<th>Situation B</th>
<th>T-1</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alone</td>
<td>Alone</td>
<td>Family</td>
<td>Alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Alone</td>
<td>Alone</td>
<td>Friends</td>
<td>Alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Alone</td>
<td>Alone</td>
<td>Classmates</td>
<td>Alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Alone</td>
<td>Family</td>
<td>Family</td>
<td>Family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Alone</td>
<td>Friends</td>
<td>Friends</td>
<td>Friends</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Alone</td>
<td>Classmates</td>
<td>Classmates</td>
<td>Classmates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Dummy variables were created in which Situation A = 0, Situation B = 1, and Situation C = 1, Situation D = 0.

### Sex Differences

Previous studies have been inconsistent regarding sex differences in trait levels of loneliness in adolescence. Some studies found girls to have higher levels of loneliness (Vanhalst, Klimstra, et al., 2012) and others found that boys had higher levels of loneliness (e.g., Hoza et al., 2000), but most studies found no sex differences in trait levels of loneliness (e.g., Bowker & Spencer, 2010; Joe-Shields et al., 2011). However, to our knowledge, sex differences have not been examined in state levels of loneliness.

In addition, several studies have shown that boys and girls experience social relations differently. For example, girls in general report receiving more provisions from their friendships, such as closeness, affection, nurturance, and acceptance (Joe-Shields et al., 2011). In addition, girls generally report receiving higher levels of parental support than boys (Bowker & Spencer, 2010). Hence, differences may exist in how boys and girls perceive their social context. Therefore, the present study examined whether state levels of loneliness differed for boys and girls, and whether boys and girls had different levels of state loneliness in different social contexts.

### The Present Study

The main goal of this exploratory study was to examine state levels of loneliness in different social contexts and different locations. First, we examined whether state loneliness differed between situations in which adolescents were alone or with company. Based on previous research and SBT, we hypothesized that adolescents would be more lonely when they were alone. Next, we examined for both situations (i.e., alone and with company) whether state levels of loneliness differed between the locations adolescents were in (i.e., home, school, and other locations). Based on the findings by Larson (1981), we expected that...
adolescents would have the highest levels of state loneliness when they were at home, compared to other situations. When adolescents were with company, we examined whether state levels of loneliness differed between different types of company (i.e., family, friends, classmates, and others). Because adolescents experience more positive affect and more positive interactions when they are with friends compared to family (Larson, 1983), we expected adolescents to have lower levels of loneliness when they are with friends, compared to other types of company. Next, we examined the temporal dynamics of social context on state loneliness. We expected that adolescents would experience highest levels of loneliness when they were alone at T, independent of whether they were alone or in company at T-1. In other words, we did not expect differences between situation A and B, which would be indicative of a habituation effect. Further, similarly to Larson (1997), we expected a relief effect when adolescents would enter the company of others at T after being alone at T-1 (i.e., lower levels of loneliness in situation D, compared to situation C). However, we expected this relief effect only for entering the company of intimate others such as family and friends, as according to SBT, non-intimate others such as classmates may not have similar benefits as intimate company (i.e., risk distribution and load sharing).

For all relations, we examined sex differences. Because previous studies found that girls value their social relations more highly than boys (Jobe-Shields et al., 2011), we expected girls to be more lonely when they were alone, compared to boys. In addition, we hypothesized girls to be less lonely than boys in the company of others, especially friends. For the temporal dynamics of state loneliness we did not have specific hypotheses regarding sex differences.

Method

Participants
Our sample consisted of 303 adolescents, aged between 13 and 16 years (M_{age} = 14.19, SD = 5.5). Of this sample, 59% was female and 97.3% was born in the Netherlands. All adolescents were in their second year of secondary school. The different types of education were well represented in the sample: 23.4% of the adolescents attended preparatory secondary school for technical and vocational training, 35.8% attended preparatory secondary school for professional education, and 40.8% attended preparatory secondary school for university. The present study was approved by the Medical Ethical Committee (CMO Arnhem-Nijmegen, 2009, No. 285). Data were collected in four schools, all in the Eastern part of the Netherlands. As this study was part of a larger project, adolescents had to consent to saliva collection for genetic analysis. Therefore, and because the momentary assessments were time-consuming for participants, adolescents and their parents had to provide active consent. Of the total group of adolescents that were contacted (N = 933), 32.5% of the adolescents agreed to participate.

Procedure
The study consisted of a baseline questionnaire and momentary assessments. The baseline questionnaire, in which demographic variables such as sex, age, and educational level were measured, was administered two to eight weeks before the start of the momentary assessments. Daily data were collected by using the Experience Sampling Method (Myin-Germeys et al., 2009), which is used to assess adolescents’ experiences in their daily living environment. Adolescents carried a smartphone on six consecutive days (including the weekend), receiving a signal at 9 random times a day, after which they had to fill out a questionnaire on the smartphone. Adolescents were instructed to turn on the smartphone when they woke up in the morning, and turn it off when they went to bed in the evening. When adolescents did not respond within two minutes, a reminding signal was emitted (with a maximum of three reminders). After adolescents filled out the questionnaire, a text message was sent to the principal investigator, making it possible to check compliance and contact adolescents when compliance was not high enough. All adolescents participated during school weeks, and adolescents had to fill out questionnaires during school hours as well. Adolescents received a reward of € 20 (i.e., about 27 US $) when they completed at least 55% of the momentary assessments (78.5%; N = 238).

Measures
State levels of loneliness. Because previous ESM studies measured loneliness by one item only (i.e., I feel lonely), we created new items to measure momentary loneliness. We used four items: lonely, isolated, left out, and abandoned. At each momentary assessment, adolescents had to rate to what extent they experienced the described emotion on a 7-point scale ranging from (1) not at all to (7) very much. We calculated Cronbach’s alpha for each momentary assessment separately, and then averaged this over all momentary assessments. This resulted in an alpha of .73. Inter-item correlations ranged from r = .40 to r = .54.

Social contexts. At each momentary assessment, adolescents rated whether they were alone or with others. When they were with others, they described in an open-ended question who that company was. These responses were coded to represent family (e.g., parents or siblings), friends, classmates, or others (e.g., team mates or teachers). To calculate the inter-rater reliability, 10% of the total number of assessments in company (N = 676) were randomly selected and coded by a different rater. This resulted in a kappa of 97 (p < .001), indicating good inter-rater reliability. To be able to examine the lagged relations between different social contexts and state loneliness, we created dummy variables representing the different types of situations (see Table 1).

Locations. In addition to social contexts, participants described in an open-ended question where they were at each assessment. These responses were coded to represent home, school, and other locations (e.g., supermarket or friends’ home). To calculate the
CHAPTER 2 LONELINESS IN THE DAILY LIVES OF ADOLESCENTS

First, we calculated descriptive statistics for state levels of loneliness and the percentage of time spent in different social contexts. State levels of loneliness were aggregated within persons. The percentages of time spent in different contexts represent the proportion of the total number of assessments adolescents spent in the different contexts.

Third, we examined whether state levels of loneliness differed between the contexts adolescents were in. Because our repeated momentary assessments (Level 1) were nested within individuals (Level 2), we conducted multilevel linear regression analyses in Mplus (Muthén & Muthén, 1998-2007). We first tested these associations for boys and girls together. However, because we expected sex differences, we subsequently analyzed the associations among the variables separately for boys and girls, by conducting multi-group analyses across sex. We examined whether the model in which the paths were allowed to differ between boys and girls had a significantly better model fit than the model in which the paths were constrained to be equal for boys and girls, using a chi-square difference test ($\Delta \chi^2$). If significant differences between boys and girls would emerge, we further compared differences between boys and girls per path, by examining whether the model fit of the model in which the path of interest was allowed to differ between boys and girls was significantly better than the model fit for the model in which all paths were constrained, also by using the chi-square difference test. In this way, significant model fit differences indicated whether the paths of interest specifically differed between boys and girls. Importantly, in all multilevel models, no missing data was imputed, as we only used the data that were available for each adolescent. For example, when an adolescent filled out 20 assessments in total, that was the number of assessments that were included in the analyses.

Next, we examined whether state loneliness differed between situations in which adolescents were alone compared to situations in which adolescents were with company, by adding a dummy variable in the model for being with company. In this way, the intercept reflected the level of loneliness when adolescents were alone, and the coefficient for the dummy variable reflected whether the level of loneliness when adolescents were in company differed from the level of loneliness when adolescents were alone. In the next model, we examined whether levels of state loneliness differed between the different locations in which adolescents were alone, by including dummy variables in the model for being alone in school versus other locations versus home. We tested two models with different reference groups (i.e., home and school, respectively), to examine all possible differences between locations. The same was done for situations in which adolescents were with company.

In the subsequent multilevel models, we tested whether state loneliness differed between situations alone and the different types of company, and between the different types of company (i.e., family, friends, classmates, and others) by adding dummy variables to the model. To examine the differences between type of company, we tested three models with different reference groups (i.e., family, friends, and classmates, respectively). Finally, we tested whether the effects of being alone or with company were dependent on their social context at the previous assessment (see Table 1), again by using multilevel modeling. In all models, state loneliness at T was the dependent variable, which was predicted by a dummy variable representing the different situations. All lagged relations were examined within days and we controlled for state loneliness at T-1. As the time elapsed between two subsequent assessments may influence the results, we controlled for this in all analyses.

Results

Descriptive Statistics

First, we calculated descriptive statistics for state levels of loneliness, for boys and girls separately (see Table 2). No differences were found between boys and girls in state loneliness. Compared to the range of the loneliness measure (i.e., 1-7), mean levels were relatively low, but comparable to levels of negative affect in other adolescent samples (Schneiders et al., 2007). Next, the percentage of time spent in different social contexts (i.e., alone, with family, with friends, with classmates, and with others) was calculated for each adolescent. As can be seen in Table 2, adolescents spent a large part of their time alone (36% for girls, 43% for boys) and with classmates (26% for girls, 27% for boys). Boys spent significantly more time alone than girls, and girls spent more time with family, friends, and others than boys.

Second, we examined correlations between state loneliness and the time spent in different social contexts (Table 3). For both boys and girls, state levels of loneliness were positively related to time spent alone, and negatively related to time spent with family.
This indicated that higher mean levels of state loneliness are related to more time spent alone, and lower mean levels of state loneliness are related to more time spent with family.

### State Loneliness in Different Social Contexts and Locations

Third, we examined whether state levels of loneliness differed between the contexts adolescents were in. We tested the unconditional model first, including only a constant and state loneliness. The intra-class correlation was .37, indicating that 37% of the variation in state loneliness occurred at the individual level (Level 2). Further, the variances in state loneliness were significant at the momentary assessment level (Level 1 variance = .27) and the individual level (Level 2 variance = .16).

Next, we examined whether state loneliness differed between situations in which adolescents were alone compared to situations in which adolescents were with company. Adolescents had significantly lower levels of state loneliness when they were with company (β = -1.38, p < .001), compared to when they were alone (β = 1.38, p < .001). No significant differences were found between boys and girls (Δχ² (1) = 0.50, p > .05).

In the next model, we examined whether states of loneliness differed between the different locations in which adolescents were alone, by including dummy variables in the model for being alone in school versus other locations versus home (Table 4). No differences in state loneliness were found between the different contexts in which adolescents were alone, and no significant differences were found between boys and girls (Δχ² (2) = 1.97, p > .05). The next step was to examine whether loneliness differed between the locations in which adolescents were with company (i.e., home, school, and other locations). Adolescents were more lonely when they were in company at school, compared to being in company at home or in other locations. No differences were found in state loneliness between being at home and being in other locations. No sex differences were found for these relations (Δχ² (2) = 1.63, p > .05).

### Table 2 Descriptive Statistics, Split for Boys and Girls

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th></th>
<th>Girls</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SD)</td>
<td>Range</td>
<td>M (SD)</td>
<td>Range</td>
<td>t</td>
<td>df</td>
</tr>
<tr>
<td>State loneliness</td>
<td>1.29 (0.39)</td>
<td>1 - 2.97</td>
<td>1.34 (0.68)</td>
<td>1 - 3.39</td>
<td>-1.49</td>
<td>282</td>
</tr>
<tr>
<td>Time alone</td>
<td>42.51 (15.76)</td>
<td>43 - 84.6</td>
<td>35.55 (14.38)</td>
<td>0 - 73.2</td>
<td>4.41***</td>
<td>282</td>
</tr>
<tr>
<td>Time with family</td>
<td>19.31 (13.03)</td>
<td>0 - 54.3</td>
<td>23.86 (13.08)</td>
<td>0 - 63.2</td>
<td>-2.88**</td>
<td>282</td>
</tr>
<tr>
<td>Time with friends</td>
<td>8.90 (7.53)</td>
<td>0 - 29.3</td>
<td>12.91 (10.61)</td>
<td>0 - 52</td>
<td>-3.73***</td>
<td>282</td>
</tr>
<tr>
<td>Time with classmates</td>
<td>26.99 (10.21)</td>
<td>0 - 54.2</td>
<td>25.30 (8.30)</td>
<td>4 - 52.6</td>
<td>1.48</td>
<td>213</td>
</tr>
<tr>
<td>Time with others</td>
<td>1.74 (2.85)</td>
<td>0 - 14.6</td>
<td>2.92 (4.41)</td>
<td>0 - 37.8</td>
<td>-2.75**</td>
<td>281</td>
</tr>
</tbody>
</table>

Note. * p < .05; ** p < .01; *** p < .001.

These parameters represent the level of loneliness for the reference group (i.e., when the dummy variable was 0). The B’s represent the difference in state loneliness between the dummy variable and the reference category.

### Table 3 Correlations Between State Loneliness and Time Spent in Different Social Contexts, Split for Boys and Girls

<table>
<thead>
<tr>
<th></th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. State loneliness</td>
<td>-</td>
<td>27**</td>
<td>-22**</td>
<td>-10</td>
<td>-02</td>
<td>.06</td>
</tr>
<tr>
<td>2. Time alone</td>
<td>-.25**</td>
<td>-</td>
<td>-61***</td>
<td>-29***</td>
<td>-29***</td>
<td>-1.5</td>
</tr>
<tr>
<td>3. Time with family</td>
<td>-22*</td>
<td>-61***</td>
<td>-</td>
<td>-30***</td>
<td>-.06</td>
<td>-11</td>
</tr>
<tr>
<td>4. Time with friends</td>
<td>-.17</td>
<td>-23*</td>
<td>-.25**</td>
<td>-</td>
<td>-27***</td>
<td>-03</td>
</tr>
<tr>
<td>5. Time with classmates</td>
<td>-.00</td>
<td>-.57***</td>
<td>-.08</td>
<td>-.05</td>
<td>-</td>
<td>-13</td>
</tr>
<tr>
<td>6. Time with others</td>
<td>.07</td>
<td>-5.0</td>
<td>-.20*</td>
<td>.01</td>
<td>.03</td>
<td>-</td>
</tr>
</tbody>
</table>

Note. Above the diagonal correlations for girls, below the diagonal correlations for boys.

* p < .05; ** p < .01; *** p < .001.

In addition, we examined whether levels of loneliness differed between situations in which adolescents were alone, compared to situations in which they were with family, friends, classmates, or others (Table 5). We found that state loneliness was lower when adolescents were with any type of company (i.e., family, friends, classmates, or others) compared to being alone. No sex differences were found for this model (Δχ² (4) = 3.96, p > .05).

### Table 4 Levels of Loneliness when Alone or in Company, Split for Differences Between Locations

<table>
<thead>
<tr>
<th></th>
<th>Situations Alone</th>
<th></th>
<th>Situations in Company</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Home</td>
<td>School</td>
<td>Other</td>
<td>Home</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.38 (03)***</td>
<td>.06 (04)</td>
<td>.01 (04)</td>
<td>1.24 (02)***</td>
</tr>
<tr>
<td>B(SE)</td>
<td>1.44 (05)***</td>
<td>-.05 (05)</td>
<td>-.09 (02)***</td>
<td>1.33 (03)***</td>
</tr>
</tbody>
</table>

Note. * p < .05; ** p < .01; *** p < .001.

These parameters represent the level of loneliness for the reference group (i.e., when the dummy variable was 0). The B’s represent the difference in state loneliness between the dummy variable and the reference category.
Next, we examined differences between the types of company adolescents were with (i.e., family, friends, classmates, and others). As can be seen in Table 5, adolescents had significantly lower levels of loneliness in situations in which they were with friends and family, compared to situations in which they were with classmates. No differences were found between the other situations. In addition, no differences were found between boys and girls ($\Delta\chi^2 (3) = 3.41, p > .05$).

<table>
<thead>
<tr>
<th>Situation</th>
<th>Alone</th>
<th>Family</th>
<th>Friends</th>
<th>Classmates</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.38 (03)**</td>
<td>-1.11 (03)**</td>
<td>-0.05 (02)**</td>
<td>-1.13 (05)**</td>
<td></td>
</tr>
<tr>
<td>B(SE)</td>
<td>-0.03 (03)</td>
<td>1.24 (02)**</td>
<td>0.09 (02)**</td>
<td>0.02 (05)</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.03 (02)</td>
<td>1.27 (02)**</td>
<td>0.06 (02)**</td>
<td>-0.01 (05)</td>
<td></td>
</tr>
<tr>
<td>B(SE)</td>
<td>-0.09 (02)**</td>
<td>-0.06 (02)**</td>
<td>1.33 (03)**</td>
<td>-0.07 (05)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Levels of Loneliness in Different Social Contexts

Note. * p < .05; ** p < .01; *** p < .001.

Temporal Dynamics of Social Contexts on State Loneliness

In the next models, we tested whether the effects of being alone or with company were dependent on their social context at the previous assessment (see Table 1). We controlled for the time elapsed between two assessments in all analyses ($M_{time}$ (in minutes) = 101.77, $SD = 77.75$). Importantly, including this variable did not change the results.

We first examined the three models (Models 1-3 in Table 1) in which situation A (two consecutive assessments in solitude) was compared with situation B (no prior solitude). No differences were found in levels of loneliness between these situations (see Table 6). This finding indicated that being alone at the current assessment has the strongest effect on loneliness, independent of whether adolescents were alone or with friends, family or classmates at the previous assessment. This is indicative of a habituation effect, as adolescents are as lonely when they are alone on two consecutive moments compared to when they are alone on one moment. In addition, no sex differences were found ($\Delta\chi^2 (2)$ ranged between 0.87 and 3.16, $p > .05$).

Next, we examined whether levels of loneliness differed between situations C (prior solitude) and D (no solitude). For the situations in which adolescents were with family at T (Model 4 in Table 1), we found higher levels of loneliness in situation C, compared to situation D. This result indicated that being alone at T-1 has a spillover effect on loneliness at T, because adolescents were more lonely in situations in which they were alone at the previous assessment (T-1), even though they were in the company of their family at the current assessment (T). No sex differences were found ($\Delta\chi^2 (2) = 1.43, p > .05$). For the situations in which adolescents were with friends at T (Model 5 in Table 1), lower levels of loneliness were found in situation C, compared to situation D. In situations in which adolescents were with friends at the current assessment, they were less lonely when they were alone at the previous assessment, compared to situations in which they already were with friends at T-1. This is indicative of a contrast effect, and more specifically, a relief effect. No significant differences were found between boys and girls ($\Delta\chi^2 (2) = 2.24, p > .05$).

Finally, for situations in which adolescents were with classmates, no differences were found between situation C and D. The model in which all paths were allowed to differ showed a significant better model fit compared to the model in which all paths were constrained ($\Delta\chi^2 (9) = 9.96, p < .05$). Therefore, we further checked whether this sex difference was due to the dummy variable representing situation C versus D, which was not the case ($\Delta\chi^2 (1) = 0.56, p > .05$), showing that for boys and girls, no differences in state loneliness were found between situation C and D.

Table 6 Model Results for Temporal Dynamics of State Loneliness

<table>
<thead>
<tr>
<th>Model</th>
<th>Situation A (alone-alone)</th>
<th>Situation B (company-alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All company</td>
<td>1.39 (03)**</td>
<td>.01 (02)</td>
</tr>
<tr>
<td>Family</td>
<td>1.39 (03)**</td>
<td>-0.03 (03)</td>
</tr>
<tr>
<td>Friends</td>
<td>1.40 (03)**</td>
<td>-0.02 (04)</td>
</tr>
<tr>
<td>Classmates</td>
<td>1.39 (03)**</td>
<td>.06 (03)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model</th>
<th>Situation C (alone-company)</th>
<th>Situation D (company-company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All company</td>
<td>-0.03 (02)</td>
<td>1.29 (02)**</td>
</tr>
<tr>
<td>Family</td>
<td>.04 (02)**</td>
<td>1.21 (02)**</td>
</tr>
<tr>
<td>Friends</td>
<td>-0.08 (03)**</td>
<td>1.26 (04)**</td>
</tr>
<tr>
<td>Classmates</td>
<td>-0.02 (04)</td>
<td>1.34 (03)**</td>
</tr>
</tbody>
</table>

Note. * p < .05; ** p < .01; *** p < .001.

1 As the only other study examining state levels of loneliness in different contexts (Larson, 1981) used only one item to measure loneliness, we checked whether our results would change when we used only that item (i.e., I feel lonely) in our analyses. Some of the results were slightly less strong, compared to the analyses with the composed loneliness scale, but the direction of results did not change. Hence the difference in measurement of state loneliness could not explain the difference in results between our study and that of Larson (1981).
Discussion

In the present study, in which the Experience Sampling Method was used, we examined state levels of loneliness in different social contexts and locations in a sample of early adolescents. Our main finding was that adolescents experienced higher levels of loneliness when they were in company at school, compared to being in company at home and other locations. These findings are not in line with previous research, in which it was found that adolescents are more lonely at home than at school (Larson, 1981). In addition, we found a spill-over effect when adolescents were alone at T-1, and with family at the consecutive assessment, which means that being alone had a lasting effect on adolescents’ levels of state loneliness. The opposite was found for being with friends; when adolescents were alone at T-1 and entered the company of friends at T, they had significantly lower levels of loneliness than in two consecutive assessments with friends. This is a contrast effect, adolescents may feel relieved that they are not alone anymore.

State Loneliness In Different Contexts

We found that when adolescents were in company, they were more lonely at school than at home and other places. A possible explanation for this finding may be that school is a context in which peers are always present, which increases the opportunities for peer rejection or negative peer interactions. In addition, going to school is obligatory, and adolescents are not free to choose their classmates, suggesting that they will also be confronted with peers they may not particularly like or who may be socially threatening or rejecting. Therefore, their levels of loneliness may be higher in school, compared to home and other locations. However, Larson (1981) found that adolescents had lower levels of state loneliness at school, which is in contrast with our results. A possible explanation for the differences in findings may be that adolescents in our sample are likely to use virtual media to stay in touch with their friends. Hence, although they are at home, they may still be interacting with their peers through text messaging or social media. In the 1980’s, these technologies were not yet available, which could explain the higher loneliness levels at home in that sample. Hence, as adolescents in our sample may have been more connected with their peers when at home, lower levels of loneliness were found at home, compared to school. In contrast, adolescents in Larson’s sample were less able to keep in touch with their friends at home, and therefore that study found that adolescents were more lonely at home than at school.

When comparing situations in which adolescents were alone with situations in which they were with different types of company, we found that adolescents were less lonely when they were in any type of company, compared to being alone, which is in line with Social Baseline Theory (Beckes & Coan, 2011). Related to this and also in line with SBT, we found that both boys and girls experienced higher levels of loneliness when they were with classmates, compared to friends and family. Being with classmates seems to be a negative situation, in which adolescents feel more lonely than in situations when they are with friends or family. This difference in state loneliness may be due to a difference in the level of closeness with family and friends versus classmates. Because classmates are peers that adolescents do not voluntarily choose to be with, the relations adolescents have with classmates are likely to be of lower quality compared to the relations they have with their friends. Therefore, adolescents may feel more lonely when they are in company that they do not necessarily have good relations with (in this case, classmates). Further, according to SBT, non-intimate company such as classmates may not have the same beneficial effects as intimate company (i.e., risk distribution and load sharing), as classmates may to some extent represent a threat to adolescents. In addition, research on peer relations has shown that children and adolescents who are rejected or victimized by their peers experience high levels of loneliness (for review, see Asher & Paquette, 2003), which could imply that adolescents who experience high levels of state loneliness with their peers are those who are rejected or victimized by their classmates. However, we did not have information on peer status or victimization in the present study. Further research is warranted to examine (a) whether the level of closeness with company in daily life affects state loneliness levels and (b) whether the level of rather objective rejection experiences affects the level of state loneliness experienced in different social contexts.

Interestingly, for both boys and girls, there were no differences in levels of loneliness between being with family or friends. Previous research (Larson, 1983) did find differences in positive affect between being with family or friends, and between being with family or peers (Silk et al., 2011) in that adolescents had higher levels of positive affect when they were with friends or peers, compared to family. When interpreting these findings in combination with the findings of the present study, it could indicate that although being with friends leads to higher levels of positive affect, being with family can fulfill the need for social relations as well as being with friends, because we found that state levels of loneliness did not differ between those situations.

Temporal Dynamics of Social Contexts on State Loneliness

Regarding the temporal dynamics of social contexts on state loneliness, we did not find differences in loneliness when being alone at two consecutive assessments (Situation A) versus being with company at T-1 and alone at T (Situation B; see Table 1 for all situations). This is indicative of habituation. Adolescents may adapt to the situation of being alone, and therefore do not feel more lonely when they are alone at two consecutive time points, compared to being alone at one time point. For both boys and girls, we found differences between two consecutive assessments in company (Situation D) and being alone at T-1 and in company at T (Situation C), depending on the type of company.

When adolescents were alone at T-1 and entered the company of friends at T, they had significantly lower levels of loneliness than in two consecutive assessments with friends. This finding can be interpreted as a relief effect. Levels of loneliness may be lower...
in situations in which adolescents enter the company of friends after solitude, because they are relieved that they are not alone anymore. These findings are in line with studies from Larson and colleagues on affect and solitude (Larson, 1997; Larson et al., 1982), in which it was found that adolescents experienced higher levels of positive affect when they entered the company of others after a period of solitude, compared to situations in which they were in company at both time points. Larson interpreted this finding as a positive after-effect of solitude, whereas we interpreted this finding as a relief effect. Hence, although our finding is similar to that of Larson, the interpretation of this finding is different. We interpreted it as a relief effect, because being alone was related to higher levels of loneliness, and likely represents a relatively negative experience for adolescents. This would also be in line with SBT, that states that being alone is a negative situation for individuals. Therefore, when adolescents feel less lonely when they enter the company of others, it seems more logical to assume relief for being in a more positive situation (i.e., with others instead of being alone) rather than a positive after effect of this (negative) situation. In addition, when the interpretation of a positive after-effect would be correct, it could be expected that this effect will be present irrespective of the company adolescents are in after having been alone. We, however, only found this effect for the company of friends, but not the company of family or classmates. We think it is more likely that for state loneliness, this finding represents a relief effect. Yet, further research is necessary to examine which interpretation is more likely.

We found opposite results for situations with family. When adolescents were alone at T-1 and with family at T, they had higher levels of loneliness than in situations in which they were with family at both time points. This is a spill-over effect, which means that being alone has a lasting effect on adolescents’ levels of state loneliness, that is still present when they are with family at the next assessment. Hence, although we did not find differences in overall levels of state loneliness between being with family and friends, these results do indicate that family and friends play a different role when adolescents were alone at the previous assessment. Family members cannot compensate the negative after effects of solitude, whereas friends seem to buffer these negative effects. This may be explained by the type of activity adolescents engage in with friends and family, as previous Experience Sampling studies have shown that interactions with friends were rated more positively than interactions with family (Larson, 1983). Therefore, entering the company of friends after a period of solitude may be more rewarding and therefore lead to greater decreases in loneliness, than entering the company of family, because the interactions are more positive. These findings might imply that adolescents who experience loneliness when alone could be advised to seek the company of their friends, as our results showed that only friends could buffer the negative effects of being alone.

Strengths and Limitations

One of the main strengths of the present study is that we used the Experience Sampling Method. In this way, it was possible to examine state levels of loneliness in adolescents’ everyday life, thereby reducing recall bias and increasing ecological validity (Myin-Germeys et al., 2009). In addition, the present study is among the first to examine state levels of loneliness in adolescence, which is a particularly important period for the development of trait levels of loneliness (Qualter et al., 2013).

Despite these strengths, some limitations have to be mentioned. First, because adolescents had to provide active consent, we may have selected a relatively healthy sample, with low levels of problem behavior. We do not have information from adolescents that declined participation, therefore, we do not know what their reasons were. Yet, the levels of loneliness in our sample are comparable to those in other community samples (Doane & Adam, 2010; Marcoen & Goossens, 1993), which indicates that we did not have a biased sample concerning levels of loneliness.

Second, we asked adolescents to report whether they were alone or in company. We did not further specify the situation in which adolescents were alone, whereas Larson (1990) states that situations in which adolescents are exchanging information with other people should be seen as situations in which they are with company. In our study, this would include situations in which adolescents were alone but talking on the phone, or involved in social networking on the Internet. However, Larson did not actually test whether there were differences in, for example, positive and negative affect between those situations. Therefore, we do not know whether this could have influenced our
results. It is important to stress however, that recent research has shown that using social media such as Facebook at a given moment was related to a decrease in wellbeing at the next moment (Kross et al., 2013), which implies that staying in touch with peers through virtual media may not necessarily be positive. As virtual media become increasingly popular, it is important to examine in future studies whether levels of state loneliness are affected by the use of virtual media. In addition, we did not measure whether adolescents chose to be alone, which may have influenced the results. Adolescents may be less lonely when they are alone because they want to be alone, compared to situations in which they did not choose to be alone. Future studies could include a question that measures whether adolescents chose to be alone. Related to this, adolescents reported where they were, but when they were at school, we could not distinguish class situations from break times. These situations may be related to different levels of state loneliness. For example, break times are typically associated with higher levels of bullying and victimization (e.g., Craig, Pepler, & Atlas, 2000). Future studies should try to distinguish between those situations, in order to obtain a more complete picture about levels of state loneliness in school.

Third, the measure of state loneliness was developed for the present study. Although it showed adequate reliability, inter-item correlations were moderate, and the measure has not been validated in other studies. Importantly, we want to stress that it may not be surprising that inter-item correlations were only moderate, as the different items represent different aspects of loneliness. For example, adolescents may feel left out when they are with peers without responding to the item 'I feel lonely', whereas they may feel more lonely and less left out when they are alone. Further research is needed to examine whether this measure proves to be valid as well. Related to this, we could not further distinguish between the experience of emotional and social loneliness, as our state loneliness measure did not capture those different aspects of loneliness. Future research could examine whether the relations between social contexts and state loneliness are different for social and emotional loneliness.

Finally, our compliance rate (i.e., 69%) is moderate compared to other ESM studies in adolescents that had higher compliance rates (e.g., Schneider et al., 2007). There may be several reasons for this difference in compliance rates. Whereas traditional ESM studies used wristwatches that emitted beeps and paper-and-pencil questionnaires, we used smartphones that emitted buzzing signals. Adolescents may have put the smartphones in their pockets or bags and therefore could have missed a buzzing signal. A beep emitted by a wristwatch may be less likely to be missed. However, we had clear reasons to use smartphones and buzzing signals. We used buzzing signals in order to minimize the disturbance in classrooms. In our opinion, it would not have been possible to convince schools to participate with multiple students at the same time, when we would have used beeps. Further, we chose to use smartphones because it made the administration of the questionnaires easier and less time-invasive for adolescents, and made it possible for us to check compliance, as we received a message when adolescents completed a questionnaire.

In addition, because the data was stored on the smartphone and messages were sent to us after each questionnaire, adolescents were not able to fill out the questionnaires all at once, which may be a problem with paper-and-pencil questionnaires. Hence, although there are some downsides to using smartphones and buzzing signals, we feel that the advantages outweigh the disadvantages.

**Conclusions**

In sum, the main findings of this exploratory study were that state loneliness is higher when adolescents were alone, compared to when they were with others. Our findings showed that school was a relatively negative environment, in that levels of loneliness were higher at school and with classmates, compared to other situations. Adolescents showed a spillover effect of solitude on state loneliness when they were alone and entered the company of family. In contrast, adolescents showed a relief effect when they were alone first and subsequently in the company of friends. These findings provide insight in the prevalence of state loneliness in early adolescence. Additional research is necessary to further disentangle the dynamics of state levels of loneliness.
Chapter 3
Trait and state levels of loneliness: Examining developmental and cultural differences in adolescence

Submitted as:
CHAPTER 3

TRAIT AND STATE LEVELS OF LONELINESS

Abstract

Previous research has mainly focused on examining trait levels of loneliness. Very little is known about state levels of loneliness and no studies have examined how and when trait lonely individuals experience state loneliness in their daily lives. Hence, the aim of the present study was to examine relations between trait and state loneliness in three different samples: early Dutch adolescents, late Dutch adolescents, and late US adolescents. Data were collected by using the Experience Sampling Method. Results provided evidence for the differential reactivity hypothesis in the total sample, as high lonely adolescents had higher levels of state loneliness in situations in which they were alone, compared to low lonely adolescents. In addition, high lonely adolescents benefited more from being with intimate company, in that their levels of loneliness decreased more in company, compared to low lonely adolescents. A developmental difference was found, in that early adolescents with high levels of trait loneliness showed no differences in state loneliness between situations alone and with non-intimate company, compared to early adolescents with low levels of trait loneliness. In the late adolescent samples, no moderation of trait loneliness was found in this relation. In sum, the present study provides important insights in the daily experiences of trait lonely people, which may provide starting points for interventions.

Introduction

Adolescence is an important period in life which is characterized by profound changes in the social domain. These social changes come into play because of intra-individual developments, such as pubertal development and brain maturation, which make adolescents increasingly able to initiate more intimate social relationships. In addition, adolescents go through important social transitions. In early adolescence, adolescents make the transition from primary school to secondary school, whereas in late adolescence, the transition to college takes place. Because of these transitions, adolescents have to establish new social relationships with peers, while reorganizing the already existing relationships with their parents and peers from before the transition. When adolescents are not successful in one of these tasks, feelings of loneliness can arise. Hence it is not surprising that feelings of loneliness are particularly present during adolescence (e.g., Heinrich & Gullone, 2006; Vanhalst, Klimstra, et al., 2012).

Loneliness is defined as the negative emotions that arise in response to a perceived discrepancy between the actual and desired quality and quantity of social relationships (Perlman & Peplau, 1981). Loneliness is typically examined as a trait, by means of questionnaires (e.g., LLCA; UCLA). Trait levels of loneliness can have severe consequences, such as depression (Cacioppo, Hughes, et al., 2006; Vanhalst, Klimstra, et al., 2012), sleep problems (Kurina et al., 2011), and heightened risk of mortality (Holt-Lunstad et al., 2010). These negative consequences highlight the importance of finding starting points for interventions to reduce feelings of loneliness. A possible way to find such starting points is by examining daily life characteristics of lonely people, as these real-life experiences may be more malleable than trait levels of loneliness. Although several studies have examined how lonely people experience their daily lives (e.g., Hawkley, Burleson, Berntson, & Cacioppo, 2003; van Roekel et al., 2013), no studies have investigated how and when lonely adolescents experience momentary feelings of loneliness in their daily lives (i.e., state loneliness). Hence, the aim of the present study was to examine relations between trait loneliness and feelings of loneliness in daily life (i.e., state loneliness), in both early and late adolescents. In addition, as social experiences may be dependent on culture, we examined these relations in samples from both the Netherlands (early and late adolescents) and the United States (late adolescents).

State Loneliness in Different Contexts

Very little is known about the experience of loneliness in daily life. To our knowledge, only three studies have examined state levels of loneliness, all in early adolescents (Larson, 1981, 1990; Larson et al., 1982). Those studies showed that adolescents experienced the highest levels of state loneliness in situations when they were alone, compared to situations in which they were with others (Larson, 1990). When considering the different locations adolescents were in, it was found that adolescents were more lonely at home,
trait and state levels of loneliness

The Present Study

The main aims of the present study were (1) to examine state levels of loneliness in different daily contexts (i.e., type of day and type of company) and (2) to examine relations between trait and state levels of loneliness in early and late adolescents, and in Dutch and American adolescents. We used the Experience Sampling Method (ESM) to examine these relations (Myin-Germeys et al., 2009). By using this method, adolescents reported on their feelings of loneliness while they were living their daily lives. Compared to more traditional methods, ESM has two important advantages: (a) recall bias is minimized and (b) ecological validity is high, because adolescents report on their feelings and social contexts while they are actually in it.

First, we investigated the differential exposure hypothesis, by examining relations between trait loneliness and the time adolescents spent in different social contexts. We hypothesized that these relations would not be significant, as previous studies examining this hypothesis in early and late adolescents did not find evidence for it (Cacioppo et al., 2003; Hawkley & Cacioppo, 2003; Larson, 1990). Second, we examined whether state levels of loneliness differed between type of day (week versus weekend) and type of company (alone versus intimate versus non-intimate company). Based on previous studies in early adolescents (Larson, 1981, 1990), we hypothesized that state loneliness would be higher on weekend days, compared to week days. In addition, we hypothesized that state loneliness would be highest in situations alone, followed by situations with non-intimate company and situations with intimate company. Finally, to examine the differential reactivity hypothesis, we examined whether trait loneliness moderated the relations between the different contexts and state loneliness. It was hypothesized that lonely adolescents in general would have higher levels of state loneliness. In addition, we expected lonely adolescents to be more negatively affected by situations spent alone or with non-intimate company, in that their levels of state loneliness would be even higher in those situations.

All relations were examined in three samples: Early adolescents from the Netherlands (from now on referred to as ‘early adolescents NL’), late adolescents from the Netherlands (from now on referred to as ‘late adolescents NL’), and late adolescents from the United States (from now on referred to as ‘late adolescents US’). We did not have specific hypotheses regarding the different samples.
CHAPTER 3 TRAIT AND STATE LEVELS OF LONELINESS

Method

Sample Characteristics

Early adolescent sample (NL). Data were collected on four high schools. The early adolescent sample consisted of 269 adolescents ($M_{age} = 14.19, SD = 0.54$), who were all in their second year of high school. Of this sample, 59% was female and 97.4% was born in The Netherlands. The different educational levels were all well represented. 22.8% of the adolescents attended preparatory secondary school for technical and vocational training, 34.8% attended preparatory secondary school for college, and 42.3% attended preparatory secondary school for university.

Late adolescent sample (NL). The late adolescent sample consisted of 223 Psychology and Educational Science undergraduate students (91% female) from the Radboud University Nijmegen, the Netherlands ($M_{age} = 19.60, SD = 1.49$). Of this sample, 77% was of Dutch origin, 21% was born in Germany, and 2% was born in another country. Most students left their parents’ home for college (65% versus 35% living with their parents) and typically lived in student homes. Almost all students were in their first year of college (96%).

Late adolescent sample (US). The US late adolescent sample consisted of 126 undergraduate students (51% female), who were screened and selected to represent the lower, middle, and upper quintile of the R-UCLA Loneliness Scale (Russell et al., 1980). Of the total sample, 83% was Caucasian, 7% African American, 7% Asian, Asian American, or Pacific Islander; 3% other or undeclared. Almost all students left their parents’ home for college (92.5%). See Hawkley et al. (2003) for a detailed description of exclusion criteria.

Procedure

Early adolescent sample (NL). High schools were contacted to participate in the present study, and when they consented, all second year adolescents were sent a letter in which information about the study was provided. When the adolescents and their parents agreed to participate, they had to return a signed consent form. All adolescents that returned a consent form could participate in the study.

The study consisted of a baseline questionnaire and the Experience Sampling period. In the baseline questionnaire, which was administered online during school hours, demographic characteristics and trait levels of loneliness were measured. Three to eight weeks after this assessment, the Experience Sampling period started. Adolescents were individually briefed about the procedure of the study one day prior to the start of the sampling period. They received a smartphone, on which a program was installed that emitted nine randomly timed signals per day, on six consecutive days (always starting on Fridays and ending on Wednesdays). Adolescents were instructed to attend to the smartphone at all times, and immediately fill out the questionnaire when they received a signal. When adolescents did not respond to a signal, another signal was emitted after two minutes, with a maximum of three reminders. After that, the questionnaire was made unavailable. It took around 3 minutes to fill out a questionnaire. Participants received a reward of € 20 (i.e., about 37 US $) when they completed at least 55% of the momentary assessments. The present study was approved by the Medical Ethical Committee Arnhem-Nijmegen. See van Roekel et al. (2013) for a more detailed description of the procedure.

Late adolescent sample (NL). All participants were recruited via an Internet sign-up program of the Behavioral Science Institute (BSI) of the Radboud University Nijmegen, the Netherlands. Participants were required to have a smartphone, as the ESM questionnaires were to be filled out on their own smartphone. The study consisted of three parts. First, participants filled out an online baseline questionnaire, in which questions about demographic characteristics and trait loneliness were included. Second, one week after administration of the baseline questionnaire, participants were invited to an introduction to the ESM study in groups of four participants, which took place in the BSI lab before the start of the momentary assessments. Participants were instructed to create a new Gmail email address for the present study and to install the Gmail app on their smartphone. This app was programmed to emit a signal whenever participants received a new email on their study email address. Participants were instructed to pause their activity when they received a new email and immediately fill out the questionnaire.

Third, the ESM data collection started one or two days after the instruction. The sampling period consisted of eleven days, with five questionnaires per day, at random time points between 10AM and 11PM on week days and between 11AM and 11PM on weekend days (resulting in 55 measurements in total). We used the program Mailchimp to send emails to participants on previously determined semi-random time points (i.e., time points were randomly chosen with an average time between time points of 160 minutes). In these emails, a link was provided to an online questionnaire. It took 3-5 minutes to fill out the online questionnaire. Participants received twelve course credits (for educational requirements) when they completed all parts of the study. The Ethical Committee of the Faculty of Social Sciences, Radboud University Nijmegen, approved the study protocols.

Late adolescent sample (US). One day prior to the Experience Sampling period, adolescents filled out a baseline questionnaire, in which demographic variables and trait levels of loneliness were measured. The Experience Sampling period consisted of 7 days with nine randomly timed beeps per day. Participants carried a programmable watch, that emitted signals between 10:00 AM and 12:00 (midnight). When participants received a signal, they were instructed to pause their activity, take out one of the paper-and-pencil diaries and fill out the questionnaire. Participants were asked to provide the time they received the beep, and the time they started and finished filling out the diary, so that it was possible to check how much time elapsed between the beep and the moment when participants filled out the questionnaire.
Measures

Trait loneliness.

Early adolescent sample (NL). Trait loneliness was measured with the peer-related subscale of the Louvain Loneliness scale for Children and Adolescents (LLCA; Marcoen et al., 1987), which consists of 12 items. Each item is rated on a 4-point scale, ranging from (1) never to (4) always. A sample item was “I think I have fewer friends than others have”. Cronbach’s alpha was .88.

Late adolescent sample (NL). Trait loneliness was assessed with the 20-item R-UCLA loneliness scale (Russell et al., 1980). Participants had to rate on a 4-point scale how often each statement was descriptive for them (1 = never, 4 = always). A sample item was “I feel in tune with the people around me”. Cronbach’s alpha was .92.

Late adolescent sample (US). Trait loneliness was assessed with the 20-item R-UCLA loneliness scale (Russell et al., 1980). Participants rated on a 4-point scale how often each statement was descriptive for them (1 = never, 4 = always). A sample item was “I feel in tune with the people around me”. Although we used a different loneliness measure in the early adolescent sample, previous research has shown that the two measures provide relatively comparable measurements of individual differences in loneliness (r = .76, Goossens et al., 2009). Hence, in order to make the measures comparable across the three samples, we standardized the trait loneliness scores within samples.

State loneliness.

Early adolescent sample (NL). State loneliness was measured with two items that were measured at all momentary assessments: “I feel lonely” and “I feel left out”. Participants filled out the extent to which they experienced these emotions on a 7-point scale, ranging from (1) not at all to (7) very much. The inter-item correlation across all measurements was .49.

Late adolescent sample (NL). State loneliness was measured with the same items as in the early adolescent sample: “I feel lonely” and “I feel left out”, which were rated on a 7-point scale ranging from (1) not at all to (7) very much. Inter-item correlation across all measurements was .47.

Late adolescent sample (US). State loneliness was measured with the same items as in the early adolescent sample: “I feel lonely” and “I feel left out”, which were rated on a 7-point scale ranging from (1) not at all to (7) very much. Inter-item correlation was .42.

Contextual predictors.

Early adolescent sample (NL). For type of day (i.e., week day versus weekend day), a dummy variable was created to represent assessments on week days (0) and assessments on weekend days (1). For the social contexts, adolescents reported at each assessment whether they were alone or with company. When they were with others, they were asked to describe who their company was, by choosing from the following categories: family, friends, significant other, classmates, teammates, strangers, others. These responses were coded to represent intimate company (i.e., family, friends, and significant other) and non-intimate company (i.e., classmates, teammates, strangers, others).

Late adolescent sample (US). Type of day and time of day were measured similarly to the early adolescent sample. For the social contexts, adolescents reported at each assessment with whom they were interacting and with whom they could be interacting. Based on these variables, we determined whether adolescents were alone or not (i.e., when they were not interacting with someone and could not be interacting with someone; they were alone), and who their company was. These responses were coded to represent intimate company (i.e., family, friends, and significant other) and non-intimate company (i.e., roommates, classmates, teachers, teammates, strangers, co-workers, neighbors, acquaintances, others).

Momentary Data Preparation

Early adolescent sample (NL). The total dataset consisted of 10,865 momentary assessments. Participants on average completed 37 momentary assessments, out of a maximum of 54 (SD = 11.12). Of the total sample (N = 303), we excluded the adolescents who had missing values on trait loneliness due to technical problems (1.65 %, N = 5). Further, 17 adolescents (i.e., 5.61 %) had less than 18 completed momentary assessments (i.e., one-third of the maximum number of assessments), which was the minimum to be included in the analyses. In addition, as some of the research questions involved only the assessments in which adolescents were with others, we excluded those adolescents (2.64 %, N = 8) who had very few assessments in which they were with others (i.e., less than 11 assessments with others). This resulted in a final dataset with 10,404 momentary assessments in 269 adolescents. We checked whether the adolescents that were included in the analyses differed from the adolescents that were excluded from the analyses on demographic characteristics and trait loneliness. No differences were found (p > .05).

Late adolescent sample (NL). The average number of assessments filled out within the time frame of 20 minutes after the signal, was 35.85 (SD = 9.18), out of a maximum of 55. From the total sample (N = 228), some participants were removed from the analyses (N = 3), because they had completed less than one-third of the total number of assessments (i.e., 18 out of 55). Further, we excluded those participants who had less than 11 assessments in company (N = 2), which resulted in a final sample of 223 participants with in total 8,117 momentary assessments. We checked whether the participants excluded from analyses (N=5) differed from those included in the analyses (N=223) on demographic characteristics (i.e., age, sex) and trait loneliness. No differences were found (p > .05).
Late adolescent sample (US). Adolescents on average filled out 50.57 assessments (SD = 11.95) out of 63 assessments. In the total sample (N = 133), some participants filled out less than one-third of the total number of assessments (i.e., less than 22 assessments), and were removed from the analyses (N = 9). All adolescents had more than 10 assessments in company. We checked whether the excluded adolescents differed from the adolescents in the analyses on demographic variables and trait loneliness. No differences were found (p > .05). The final dataset consisted of 6,066 momentary assessments in 126 adolescents.

Plan of Analysis
Because our momentary assessments (Level 1) were nested within individuals (Level 2), we used multilevel regression analysis. The main advantages of multilevel analysis are that it does not require participants to have data on each assessment and it controls for the dependency of the data. In multilevel analysis, predictors can be entered at each level, making it possible to examine how situational characteristics (i.e., Level 1) as well as individual characteristics (i.e., Level 2) are related to state levels of loneliness. In addition, it is possible to examine cross-level interactions, so that we can examine whether trait levels of loneliness (Level 2) moderate the relations between situational characteristics and state levels of loneliness (both Level 1 variables).

In the present study, we first calculated descriptive statistics in all samples. For state loneliness, scores were aggregated within persons to represent a mean score calculated over all assessments. In order to examine correlations between trait loneliness and the percentage of time spent in different (social) contexts, we calculated how many assessments adolescents spent alone, with intimate company and with non-intimate company, relative to the total number of assessments. The correlations were calculated separately in the three samples, and correlation coefficients were compared between samples by using Fisher r-to-z transformations.

Next, we conducted multilevel analyses to examine our research questions. As was mentioned earlier, trait and state loneliness measures were standardized within samples so that we were able to compare effects between samples. In all models, we first examined the relations in the total sample, and subsequently used multigroup analyses to examine whether the results differed between the three samples. We did this by examining whether the model fit ($\chi^2$) of the model in which the paths of interest were allowed to differ between samples was significantly better than the model fit of the model in which the paths of interest were constrained to be equal across samples. When significant differences were found, we further examined on which relations the samples differed by comparing the model fit between the multigroup model in which each path was constrained, and the multigroup model in which the path of interest was allowed to differ between samples. When significant differences were found between the samples for the path of interest, separate models were run to further examine these differences between early adolescents (NL) versus late adolescents (NL), to examine age-related differences, and between late adolescents (NL) versus late adolescents (US) to examine cultural differences.

First, we tested an initial model without predictors. Second, we examined the relation between trait and state levels of loneliness by adding trait loneliness as a Level 2 predictor. For all next models, we first examined the relations between the Level 1 predictors and state loneliness, and in a subsequent model, the cross-level interaction with trait loneliness was entered to examine whether trait loneliness moderated the relations between the Level 1 predictors and state loneliness. Third, we examined whether the type of day (week versus weekend) was related to state loneliness, by adding the dummy variable representing type of day to the model (with week days as the reference category). Fourth, the effects of social contexts were examined. Differences in state loneliness between situations alone and with company were examined by adding a dummy variable representing situations alone (score 0, reference category) versus situations with company (score 1). To examine differences in state loneliness between intimate and non-intimate company, a dummy variable was included with intimate company as the reference category. Finally, differences between being alone versus intimate and non-intimate company were examined. Assessments in which adolescents were alone were used as the reference category, and dummy variables representing intimate and non-intimate company were included. In all models, we controlled for sex.

Results

Descriptive Statistics
First, mean levels of trait and state loneliness were calculated (see Table 1). The mean levels of trait loneliness in early and late adolescents were similar to those found in other Dutch community samples (e.g., Van Roekel, Engels, Verhagen, Goossens, & Scholte, 2010), but slightly lower than those found in adolescent samples from the USA (e.g., Fiori & Conseedine, 2013; Mounts et al., 2006). Mean levels of trait loneliness in both late adolescent samples did not significantly differ from each other ($F(355) = -0.361$, p > .05). Mean trait loneliness levels could not be compared between the early and late (NL) samples, as different measures were used. State levels of loneliness were relatively low, compared to the range (1-7 in early and late NL samples, 1-5 in late US sample). The correlation between trait and state loneliness was significant in all three samples, indicating that higher levels of trait loneliness were associated with higher levels of state loneliness (see Table 2). These correlations were similar in all three samples ($\rho$’s ranged from −.46 to −.04, $\rho$’s > .05).

Regarding the time spent in different contexts, it was found that compared to the late adolescent (US) sample, early and late adolescents (NL) spent more time alone (Table 1). No significance difference was found between early and late adolescents (NL). Late adolescents (NL) spent more time with intimate company than late adolescents (US) and...
early adolescents (NL). Finally, the time spent with non-intimate company significantly differed between the three samples; late adolescents (NL) spent the least time with non-intimate company, followed by early adolescents (NL), and late adolescents (US) spent most time with non-intimate company.

To investigate the differential exposure hypothesis, correlations between trait levels of loneliness and time spent in different contexts were examined (Tables 2 and 3). For early adolescents (NL) and late adolescents (US), no significant correlations were found between trait loneliness and the time spent in different contexts. For the late adolescents (NL), a small correlation was found between trait loneliness and time spent with intimate company, in that adolescents with higher levels of loneliness spent less time with intimate company. When comparing these correlations between the samples, no differences were found between the early adolescent (NL) and late adolescent (US) samples (z-scores ranged between -0.07 and 1.1, p’s > .05), and no differences were found between the late adolescent (NL) and the late adolescent (US) samples (z-scores ranged between -0.90 and 0.54, p’s > .05). The correlation between time spent with intimate company and loneliness and the correlation between time spent with non-intimate company and loneliness did differ between early adolescents (NL) and late adolescents (NL).

### Model Results

First, we tested a model without predictors to estimate intra-class correlations (ICC) for state loneliness. In all samples combined, the ICC was .36, indicating that 36% of the variance in state loneliness can be explained by individual predictors. Next, trait loneliness was entered in the model. Trait levels of loneliness were significantly and positively related to state levels of loneliness in the total group (β = .21, SE = .03, p < .001). No differences were found between the three groups (Δχ² = 2.03, df = 4, p > .05).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Early adolescents NL</th>
<th>Late adolescents NL</th>
<th>Late adolescents US</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (SD)</td>
<td>17.67 (5.34)</td>
<td>35.88 (9.40)</td>
<td>35.12 (9.76)</td>
</tr>
<tr>
<td>N</td>
<td>269</td>
<td>223</td>
<td>126</td>
</tr>
<tr>
<td>Range</td>
<td>12-48</td>
<td>20-80</td>
<td>10-80</td>
</tr>
<tr>
<td>F</td>
<td>35.12</td>
<td>35.73</td>
<td>35.12</td>
</tr>
<tr>
<td>Percentage alone</td>
<td>37.23 a</td>
<td>37.47 a</td>
<td>29.46 a</td>
</tr>
<tr>
<td>Percentage intimate</td>
<td>34.25 a</td>
<td>52.30 a</td>
<td>35.13 a</td>
</tr>
<tr>
<td>Percentage non-intimate</td>
<td>28.52 a</td>
<td>10.12 b</td>
<td>35.41 a</td>
</tr>
</tbody>
</table>

Note. Means are compared horizontally. Mean levels with similar superscripts do not significantly differ from each other (a, b). Mean levels with different superscripts significantly differ from each other (a, b, c).

### Table 2

Correlations Between Model Variables in Early Adolescent (NL) Sample

<table>
<thead>
<tr>
<th></th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sex</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. Age</td>
<td>-.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Trait loneliness</td>
<td>.20 **</td>
<td>-.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. State loneliness</td>
<td>.10</td>
<td>-.01</td>
<td>.32 ***</td>
<td>-</td>
</tr>
<tr>
<td>5. Percentage alone</td>
<td>-.25 ***</td>
<td>.07</td>
<td>.00</td>
<td>.14 *</td>
</tr>
<tr>
<td>6. Percentage intimate</td>
<td>.29 ***</td>
<td>-.06</td>
<td>.05</td>
<td>-.18**</td>
</tr>
<tr>
<td>7. Percentage non-intimate</td>
<td>-.04</td>
<td>-.02</td>
<td>-.08</td>
<td>.04</td>
</tr>
</tbody>
</table>

Note. * p < .05; ** p < .01; *** p < .001.
Type of day.

Next, we examined whether type of day was related to state loneliness. In the total group, a significant relation was found between type of day and state loneliness, in that adolescents experienced higher levels of state loneliness during the week ($B = -0.06$, $SE = 0.02$, $p < 0.01$). However, when we compared the constrained model with the unconstrained model, significant differences were found ($\Delta \chi^2 = 10.40$, $df = 4$, $p < 0.05$) between the three groups, specifically for the relation between type of day and state loneliness ($\Delta \chi^2 = 6.94$, $df = 2$, $p < 0.05$). The relation between type of day and state loneliness was significant for the early adolescent (NL) sample ($B = -0.07$, $SE = 0.03$, $p < 0.001$) and for the late adolescent (NL) sample ($B = -0.10$, $SE = 0.02$, $p < 0.001$), but not for the late adolescent (US) sample ($B = 0.02$, $SE = 0.03$, $p > 0.05$).

In order to examine whether the differences in this relation were significant between the samples, we compared this relation between early adolescents (NL) versus late adolescents (NL), and between late adolescents (NL) and late adolescents (US). No differences were found between early adolescents (NL) and late adolescents (NL) ($\chi^2 = 0.37$, $df = 1$, $p > 0.05$). The differences between late adolescents (NL) and late adolescents (US) were significant ($\chi^2 = 6.40$, $df = 1$, $p < 0.05$). This indicates that the above mentioned findings differ between the late adolescent (US) sample and the late adolescent (NL) sample. That is, this reflected a cultural rather than developmental effect.

Next, we examined whether trait loneliness moderated these relations. In the total sample, this interaction was not significant ($B = -0.03$, $SE = 0.02$, $p > 0.05$). When we compared the constrained model with the unconstrained model, no significant differences were found ($\Delta \chi^2 = 12.99$, $df = 8$, $p > 0.05$), indicating that there were no differences in relations across the samples. Hence, trait loneliness does not moderate the relation between type of day and state loneliness in any of the samples.

Type of company.

First, we examined the relation between the dummy variable for being alone versus being in company and state levels of loneliness. In the total sample, state levels of loneliness were higher in situations alone than in situations with others ($B = 0.21$, $SE = 0.02$, $p < 0.001$). However, multigroup analyses showed that the model differed for the three samples ($\Delta \chi^2 = 14.61$, $df = 4$, $p < 0.01$), and that this difference was due to the path representing the relation between alone versus company and state loneliness ($\Delta \chi^2 = 9.78$, $df = 2$, $p < 0.01$). Further comparisons between samples showed that this relation was stronger for late adolescents (NL) ($B = 0.28$, $SE = 0.03$, $p < 0.001$), compared to early adolescents (NL) ($B = 0.19$, $SE = 0.03$, $p < 0.001$; $\Delta \chi^2 = 5.19$, $df = 1$, $p < 0.05$) and to late adolescents (US) ($B = 0.15$, $SE = 0.03$, $p < 0.01$; $\Delta \chi^2 = 8.62$, $df = 1$, $p < 0.05$). In sum, these findings indicate that late adolescents (NL) showed the greatest difference in state loneliness between situations alone and in company, whereas this difference is smaller, though significant, in the early adolescent (NL) and late adolescent (US) samples.

Next, we examined whether trait loneliness moderated this relation. In the total sample, this interaction was significant ($B = 0.05$, $SE = 0.02$, $p < 0.05$). When we compared the
unconstrained model with the constrained model, significant differences were found ($\Delta \chi^2 = 19.81, df = 8, p < .05$), that were specific to the interaction path ($\Delta \chi^2 = 8.46, df = 2, p < .01$). Further, when we compared the results between early adolescents (NL) and late adolescents (NL), a significant difference was found between the samples ($\Delta \chi^2 = 7.25, df = 1, p < .05$), whereas no difference was found between the two late adolescent samples ($\Delta \chi^2 = 0.11, df = 1, p > .05$). For early adolescents, trait loneliness did not moderate the relation between alone versus company and state loneliness ($B = -.10, SE = .03, p > .05$), whereas in late adolescents (NL) ($B = 10, SE = .03, p < .01$) and in late adolescents (US) ($B = 0.06, SE = .03, p < .05$), the interaction was significant. As can be seen in Figure 1, trait lonely adolescents in both late adolescent samples had higher levels of state loneliness when alone than non-lonely adolescents, and showed greater decreases in state loneliness when they were with others. These results suggest a developmental, rather than cultural, effect.

In the next model, we included a dummy variable representing situations with intimate and non-intimate company. This variable was significantly related to state loneliness in the total sample ($B = 11, SE = .02, p < .001$) and this model did not differ between the three samples, but not in which relations these differences exist. Therefore, we further tested whether this difference was specific for the interaction with intimate or non-intimate company. In the final model, we examined differences in state loneliness between situations alone versus situations with intimate or non-intimate company, by including dummy variables representing intimate and non-intimate company (i.e., with situations alone as the reference group). In the total sample, we found that state loneliness was lower in situations with intimate company ($B = -.14, SE = .02, p < .001$) and non-intimate company ($B = -.14, SE = .02, p < .001$), compared to situations alone. The unconstrained model did not significantly differ from the constrained model ($\Delta \chi^2 = 7.66, df = 6, p > .05$), showing that the findings did not differ across samples.

![Figure 1](image1.png)

**Figure 1** Moderation of trait loneliness in the relation between type of company (alone versus company) and state loneliness in both late adolescent samples.

Subsequently, we investigated whether trait loneliness moderated these relations. For situations alone versus intimate company, the interaction with trait loneliness was significant in the total sample ($B = -.08, SE = .02, p < .001$). For situations alone versus non-intimate company, no moderation of trait loneliness was found in the total sample ($B = -.01, SE = .02, p > .05$). When we tested whether this model differed across samples, a significant difference was found ($\Delta \chi^2 = 23.27, df = 12, p < .05$), showing that there are differences between the samples, but not in which relations these differences exist. Therefore, we further tested whether this difference was specific for the interaction with situations alone versus intimate company, which was not significant ($\chi^2 = .094, df = 2, p > .05$). This indicated that the significant interaction that was found, did not differ between samples. As can be seen in Figure 3, being alone or with intimate others has a
bigger impact on lonely adolescents, compared to non-lonely adolescents. Further, being with an intimate other lowers the level of state loneliness in high trait lonely adolescents to the level observed in non-lonely adolescents when they are alone.

Figure 3  Moderation of trait loneliness in the relation between type of company (alone versus intimate) and state loneliness in the total sample.

Next, we examined whether the differences between samples were due to the interaction of trait loneliness and situations alone versus non-intimate company, which was the case ($\Delta \chi^2 = 6.93$, $df = 2$, $p < .05$). Hence we further examined this relation in the different samples. It was found that the interaction was different for early adolescents (NL) compared to late adolescents (NL) ($\Delta \chi^2 = 4.01$, $df = 1$, $p < .05$). No difference was found between late adolescents (NL) and late adolescents (US) ($\Delta \chi^2 = 0.28$, $df = 1$, $p > .05$). The results in the different samples showed that the interaction was significant only in the early adolescent sample ($B = .08$, $SE = .04$, $p < .05$). For late adolescents (NL) ($B = .03$, $SE = .04$, $p > .05$) and late adolescents (US) ($B = .06$, $SE = .04$, $p > .05$), no interaction was found, indicating that the relation between alone versus non-intimate company and state loneliness does not differ for high and low trait lonely adolescents. For early adolescents (NL), lower levels of state loneliness were found in situations with non-intimate company compared to situations alone for low lonely adolescents (see Figure 4). For high lonely adolescents, no difference in state loneliness was found between situations alone and with non-intimate company, indicating that high lonely adolescents experienced similar levels of state loneliness when alone and when with non-intimate company.

Figure 4  Moderation of trait loneliness in the relation between type of company (alone versus non-intimate) and state loneliness in the early adolescent (NL) sample.

Discussion

In the present study, we sought to examine relations between trait and state levels of loneliness in three different samples. We found support for the differential exposure hypothesis only in the late adolescent (NL) sample in that higher levels of trait loneliness were related to less time spent with intimate company. The differential reactivity hypothesis was applicable to all samples; adolescents high in loneliness had higher levels of state loneliness when they were alone, and decreased more in state loneliness in situations with intimate company, compared to adolescents low in loneliness. These findings show that lonely adolescents responded more negatively to being alone, but found more relief in intimate company, compared to non-lonely adolescents.

Type of Day and State Loneliness

In early adolescents (NL) and late adolescents (NL), we found that state loneliness was highest during week days, compared to weekend days. In the late adolescent (US) sample, no differences were found in state loneliness between week and weekend days. The finding in the Dutch samples may be explained by the variety of choice adolescents have in who they spent their time with during week days and weekend days. During week days, both early and late adolescents may be obligated to go to school or follow courses, study, or work, and hence they have less choice in who their company is. During weekends, however, they can choose how and with whom they want to spend their leisure time, and therefore may be less lonely at those times. The difference in findings between the two late adolescents samples may be explained by differences between the two countries.
Although late adolescents in both samples may have less choice of their company during week days, it is likely that there is a difference between the US and NL sample in how they spent their weekends. In the late adolescent (NL) sample, even though adolescents may have moved out of their parents’ home, they typically spent their weekends at their parents’ home, meeting with their friends from high school. Hence, their levels of loneliness may be lower during weekends, as they spent more time with their family and friends during those times. For the late adolescent (US) sample, the distances between college and their parents’ home are often larger, making it difficult for adolescents to return to their hometown every weekend. Hence, levels of loneliness may not differ between week and weekend days in this sample, as they stay in the same environment.

No moderation of trait loneliness was found in the relation between type of day and state loneliness, indicating that the relations between type of day and state loneliness do not differ between low and high trait lonely adolescents.

**Type of Company and State Loneliness**

The findings regarding state loneliness in different social contexts were remarkably similar in all three samples. Adolescents experienced the highest levels of loneliness when they were alone versus company and lower levels of loneliness when they were with intimate company versus non-intimate company. These findings highlight the importance of intimate company such as family and friends in reducing feelings of loneliness in adolescents.

The only finding that differed between the samples was that late adolescents (NL) experienced a greater difference in state loneliness between situations alone and with company, whereas they showed greater increases in state loneliness when they were alone than the other two samples. This finding may possibly be due to the living situation of the late adolescents (NL), in that the majority of the sample moved out of their parents’ home, during week days. For these adolescents, being alone in a new city during week days, may be particularly predictive for loneliness, as they may not yet have a social network on which to fall back.

**Differential Reactivity Hypothesis: Moderation of Trait Loneliness**

In the present study, we examined the differential reactivity hypothesis by analyzing whether trait lonely adolescents showed different responses to social contexts than trait non-lonely adolescents. Most of the results were similar in all samples and in line with the differential reactivity hypothesis. We found that trait lonely adolescents experienced a greater difference in state loneliness between situations with intimate and non-intimate company and greater differences in state loneliness between situations alone and with intimate company. These findings indicate that especially for lonely adolescents, being with intimate company is a rewarding and positive situation, in that they experience the lowest levels of state loneliness. These findings are in line with previous studies in early adolescents (van Roekel et al., 2013) that showed that lonely adolescents were more rewarded by higher levels of positive company than non-lonely adolescents. These findings combined may indicate that lonely people benefit more from being with intimate company, or others that they perceive positively. Importantly, similar results were found in late adolescents with depressive symptoms, in that adolescents with more depressive symptoms reported greater decreases in negative affect and greater increases in positive affect when they perceived their company as more intimate (Brown, Strauman, Barrantes-Vidal, Silvia, & Kwapil, 2011).

However, it should be mentioned that despite the greater decreases in state loneliness when with intimate company in lonely adolescents, the levels of state loneliness were still higher in the lonely group, compared to the non-lonely group.

The finding that lonely adolescents have the highest levels of loneliness when alone, compared to intimate company, may also indicate that lonely adolescents use their time alone less constructively or more negatively than non-lonely adolescents. Previous studies have shown that trait loneliness is positively related to rumination (Vanhalst, Luyckx, Raes, & Goossens, 2012), that is, the repetitively and passively focusing on symptoms of distress (Nolen-Hoeksema, 1991), which in turn may increase negative emotions. As rumination may be a particularly solitary experience, it could be that lonely people ruminate more while they are alone, and therefore have higher levels of state loneliness. Hence, further research should focus on how adolescents spent their time when they are alone or with others, and whether these activities affect their levels of state loneliness.

Importantly, for two relations we found different results for the early adolescent (NL) sample, compared to the two late adolescent samples. For both late adolescent samples, we found that trait lonely adolescents had greater differences in state loneliness between situations alone and with company (i.e., intimate and non-intimate combined). For early adolescents, no moderation of trait loneliness was found, indicating that high and low lonely adolescents responded similarly to situations alone and with company. However, for situations alone compared to non-intimate company, we found moderation of trait loneliness in the early adolescent sample only, in that high lonely adolescents showed no difference in state loneliness between situations alone and with non-intimate company, whereas low lonely adolescents did have lower levels of state loneliness when with non-intimate company. This finding may also explain why we did not find an interaction in this sample for situations alone versus company, because adolescents high in loneliness showed no differences in state loneliness between situations alone and non-intimate company, whereas they showed greater differences in state loneliness between situations alone and intimate company. These two findings may equal out any effects of trait loneliness when intimate and non-intimate company are examined together, which was the case when we compared situations alone with situations in company.

Our findings show that early adolescents who score high on loneliness do not benefit from being with non-intimate others and underscore the importance of the quality over the quantity of interpersonal relationships in combatting loneliness (Cacioppo et al., 2000,
Hawkley et al., 2008). Instead, they showed similar levels of state loneliness in situations alone and with non-intimate company. This non-intimate company most often consisted of classmates, as those are the people they typically are with during school hours. As they do not choose to be with classmates, these situations may be particularly negative for adolescents high in loneliness, because there are more opportunities for rejection and negative peer experiences in those situations. Therefore, these adolescents may be as lonely in those situations with non-intimate company as they are in situations alone. Further, classmates may represent a more negative situation for early adolescents compared to late adolescents, as adolescents in high school spent a lot of time with those classmates and classes may be relatively small, whereas in late adolescence, lectures are often given in large groups, and adolescents can remain relatively anonymous. Hence, social rejection by non-intimate company may be less likely in late adolescents than in early adolescents.

In addition to this, we do not know whether it is only the perception of lonely adolescents that is more negative, or whether their experiences with others are also objectively more negative than the experiences of non-lonely people. For example, it may be that lonely adolescents in the early adolescent (NL) sample experience high levels of state loneliness with non-intimate company because they actually are more rejected or left out by others than low lonely adolescents. Further research is necessary to examine whether lonely adolescents perceive their environment more negatively and therefore are more lonely in certain situations, or whether those situations actually are more negative for lonely adolescents. A possible way to examine this may be by using naturalistic observations. In a study on children, Qualter et al. (2002; 2007) used playground observational data to examine differences between the experiences of lonely and non-lonely children. As early adolescents also spent most of their time in school, it would be possible to observe the interactions of lonely versus non-lonely children during breaks. For example, the amount of positive versus negative interactions during breaks could be compared between high and low lonely adolescents. This could provide important insights in the actual social contexts of lonely adolescents.

In sum, the findings on moderation of trait loneliness in the relations between social contexts and state loneliness are in line with the differential reactivity hypothesis, in that our findings show that trait lonely adolescents respond differently to social contexts than non-lonely adolescents. In most cases, our findings imply that trait lonely adolescents respond more negatively to being alone and being with non-intimate company; in that their state levels of loneliness are highest in those situations. On the other hand, we also found that trait lonely adolescents benefited most from being with intimate company, as their levels of state loneliness were lowest in those situations. However, although they may have benefited more from those situations, it has to be mentioned that their levels of state loneliness remained higher than the levels in non-lonely adolescents, indicating that they were not able to down regulate their feelings of loneliness to normative levels.

**Differential Exposure Hypothesis**

Although the three samples were found to respond relatively similarly when we tested the differential reactivity hypothesis, differences across the samples were found when we investigated the differential exposure hypothesis. For instance, the greatest difference was that late adolescents (NL) spent more time with intimate company than early adolescents (NL) and late adolescents (US), and late adolescents (US) spent more time with non-intimate company compared to the other groups. As was mentioned earlier, a possible explanation for the higher time spent with non-intimate company and lower time spent with intimate company in the late adolescent (US) sample could be the difference in living situation between the two late adolescent samples, as half of the late adolescent (NL) sample still lived at their parents’ home, whereas the majority of the US sample lived on campus. This could imply that the late adolescents (NL) are in general with intimate company when they are at home (i.e., their parents or siblings), whereas the late adolescents (US) are more likely to be with roommates when they are at home, who are defined as non-intimate company in the present study.

We did not find evidence for the differential exposure hypothesis in the early adolescent (NL) sample and the late adolescent (US) sample, whereas we did find support for this hypothesis in the late adolescent (NL) sample. However, we should acknowledge that this correlation in late adolescents (NL) did not differ from the correlation in late adolescents (US), and that the correlation was small. As the late adolescent (US) sample was smaller ($N = 126$) than the late adolescents (NL) sample ($N = 223$), it could be that we did not find this correlation in the US sample because we did not have enough statistical power in that sample. This particular finding in late adolescents may be due to the living situations in these samples, as most of these adolescents moved out of their parents’ home. It could be that these adolescents became more lonely because they spent less time with intimate company, due to their migration. However, we do not know the direction of effects, so it could also be that trait lonely adolescents experience less positive environments because they spent less time with intimate company. Therefore, we cannot conclude that these lonely adolescents were exposed to positive situations less frequently. Further research is necessary to disentangle the causality in this relation. For example, adolescents who move out of their parents’ home could report on their loneliness levels and with whom they spent their time on multiple occasions right before they move out and up to a year later, which would make it possible to examine whether their lowered time spent with intimate others precedes increases in loneliness, or whether they experience loneliness and as a consequence spent less time with intimate others.

**Strengths and Limitations**

The main strength of the present study is that we used the Experience Sampling Method, which made it possible to examine loneliness in the actual daily lives of adolescents. In addition, because we used data from three different samples, we were
able to study differences and similarities across different ages and cultures. However, some limitations need to be addressed as well.

First, some methodological issues have to be considered. To measure state loneliness, we used two items, that is, ‘lonely’ and ‘left out’. As was mentioned in the Method section, the inter-item correlations were relatively low in all three samples, which might implicate that we did not measure the same concept with those two items. However, from a theoretical point of view it seems plausible to use both items, as they represent different aspects of loneliness.

Related to this, there were some differences in the measurement of trait and state loneliness between the three samples. In the early adolescent sample, we used the Leuven Loneliness Scale for Children and Adolescents, as this questionnaire is developed for younger children and therefore suitable for early adolescents. In both late adolescent samples, we used the Revised-UCLA loneliness scale, which is typically used in late adolescent and older adult samples (Russell et al., 1980). Although these are different scales, we do think they measure the same construct, as the two scales correlated highly with each other in a student sample (r = .76; Goossens et al., 2009). Additionally, we tried to overcome this difference in measurement by standardizing the scale scores within samples. Therefore we think that this difference in measurement did not significantly influence the results. For state loneliness, we used the same items in all three samples, but the response scale differed between samples. In the Dutch samples, these items were rated on a 7-point scale, whereas in the US sample, the items were rated on a 5-point scale. Again, we standardized the state loneliness measures within samples, so that we had comparable measures between the samples. Still, the differences in response scale could have influenced how adolescents filled out the items. This should be taken into consideration when interpreting the results.

Additionally, there were some differences in how the social contexts were measured. In the early adolescent sample, we used open ended questions in which adolescents had to describe who their company was, whereas for both late adolescent samples, multiple-choice answers were provided. As we did not measure how close participants were with their company in all samples, we had to divide the social contexts in intimate versus non-intimate company based on the objective categories (i.e., family, friends, classmates) rather than on subjective experiences (i.e., how close or intimate participants were with their company). Although this means that the categories were objectively the same in all samples, there may have been differences in how close participants were with their company. For example, roommates may be considered as intimate company for some adolescents, whereas other adolescents may not experience close relationships with their roommates. However, based on our findings, we are confident in our categorization, as the levels of state loneliness adolescents experienced in the different social contexts were as expected (that is, higher loneliness in non-intimate company compared to intimate company). Further research is necessary to examine whether the objective categorization is in line with subjective levels of intimacy, and whether this affects the levels of state loneliness that are experienced in those subjective contexts.

In addition to these methodological issues, there are some limitations concerning the samples in the present study. We used normative adolescent samples, in which no chronic levels of loneliness were measured. In the late adolescent (US) sample, the adolescents were selected based on low, middle, and high scores on loneliness, but still the mean levels of loneliness in that group were relatively low, and similar to the mean levels in the late adolescents (NL) group. Although it is important to examine relations between trait and state loneliness in normative samples, it provides important information about how and when adolescents experience state levels of loneliness, further research should include samples with chronic levels of loneliness, as they may have different daily social experiences. For example, Qualter et al. (2013) only found hypervigilance to social threat in chronically lonely children, which could indicate that this group may have particularly negative social experiences in daily life as well. Further, the late adolescent (NL) sample consisted mainly of females, and both late adolescent samples consisted exclusively of highly educated adolescents (i.e., college students). Samples that are more balanced in terms of sex and educational levels may provide results that are generalizable to the general population.

Conclusion

In sum, our findings indicate that in general, trait lonely adolescents experience higher levels of state loneliness when alone, but also benefit more from being with intimate company than non-lonely adolescents, as their levels of state loneliness decrease more when with intimate company. Further, developmental differences were found. For late adolescents, trait loneliness did not moderate the relation between state loneliness and being alone versus with non-intimate company, whereas this was the case for early adolescents; lonely early adolescents did not benefit from being with non-intimate company, as their levels of state loneliness in situations with non-intimate company did not differ from their levels of loneliness in situations alone.
Chapter 4
Loneliness, affect, and adolescents’ appraisals of company: An Experience Sampling Method (ESM) study

Published as:
CHAPTER 4

LONELINESS, AFFECT, AND ADOLESCENTS’ APPRAISALS OF COMPANY

Abstract

The aims of the present study were (a) to examine relations between baseline levels of loneliness and momentary affect and perceptions of company, and (b) to test responses to perceived social threat and lowered reward response to positive stimuli in relation to loneliness in adolescents. Data were collected among 278 adolescents ($M_{age} = 14.19$, 59% girls) by using the Experience Sampling Method. Baseline loneliness was related to affect and appraisals of company. Findings revealed greater responses to social threat, in that adolescents with higher levels of baseline loneliness were more negatively affected by negative company. For the lowered reward response we found opposite effects: adolescents high in loneliness were more positively affected by positive company.

Introduction

The association between loneliness and affect is well-documented in many age groups (e.g., Ernst & Cacioppo, 1999), but this association has rarely been examined in adolescents. This is an important gap in research on loneliness, especially because adolescence is a turbulent period in which affect is highly variable (Larson, Moneta, Richards, & Wilson, 2002). In addition, studies on loneliness have examined this relation cross-sectionally, by measuring mood at one point in time. Because affective states are highly variable and context-dependent (e.g., Larson, Csikszentmihalyi, & Graef, 1980), it is important to measure affect by using momentary assessments (i.e., using multiple measures a day, for several days). Therefore, the present study examined relations between loneliness and affect in adolescence by using momentary assessments. In addition, the present study examined responses to perceived social threat and lowered reward response to positive stimuli in relation to loneliness (Cacioppo & Hawkley, 2009) in adolescents, which has not been done before.

Loneliness and its Negative Consequences

Human behavior is driven by a fundamental need to form and maintain a certain quantity of intimate and stable relationships, also called the need to belong (Baumeister & Leary, 1995). Being unable to fulfill the need to belong can evoke feelings of loneliness, when a discrepancy is experienced between the desired and actual quality and quantity of one’s social relations (Perlman & Peplau, 1981).

Chronic feelings of loneliness can have serious consequences. Loneliness is associated with psychological problems such as schizophrenia (Heinrich & Gullone, 2006), suicidal ideation (Jones, Schinka, van Dülmen, Bossarte, & Swahn, 2011), low self-esteem, social anxiety (Mahon et al., 2006), depression (Vanhalst, Luyckx, Teppers, & Goossens, 2012), and with serious physical health consequences, such as sleep deprivation, cardiovascular disease, and even increased mortality (Hawkley et al., 2010).

Loneliness in Adolescence

Loneliness is particularly present in adolescence (Heinrich & Gullone, 2006). Longitudinal studies have shown that early adolescents have the highest levels of loneliness and these levels slowly decrease throughout the course of adolescence (van Roekel, Scholte, Verhagen, Goossens, & Engels, 2010). The high levels of loneliness in early adolescence may not be surprising, because adolescence is a period in which the transition to high school takes place, peers become increasingly important, and adolescents grow to have greater expectations of their social relationships (Parkhurst & Hopmeyer, 1999), which may not always be fulfilled. Considering both the serious consequences of loneliness and the high prevalence in early adolescence, the present study will focus on loneliness in early adolescents.
Loneliness, Mood, and Perception of Social Interactions: One-Time Assessments

In cross-sectional studies, loneliness is typically associated with characteristics that are indicative of high negative affect and low positive affect. For example, loneliness is highly correlated with depressive feelings, which comprise both negative feelings (e.g., sad, unhappy), but also diminished positive feelings (i.e., anhedonia) (Forbes, Williamson, Ryan, & Dahl, 2004). In addition, lonely people are often also high in neuroticism, which is related to negative affect (Larsen & Ketelaar, 1991). Furthermore, cross-sectional studies in young adults showed that lonely people experienced higher levels of negative affect, lower levels of positive affect (Aanes, Mittelmark, & Hetland, 2009), Joiner, 1997, Mehrabian & Steff, 1995), and more negative perceptions of interaction quality (Duck et al., 1994), interaction partners (Jones, Freeman, & Goswick, 1981), and of close others (Tsai & Reis, 2009).

However, all of these studies measured affect and perceptions of others and interactions at one-time point. In this way, participants retrospectively rated how they felt in the last period (most often in the last two weeks), or had to rate how they perceived others in general. This may constitute a suboptimal way to measure these constructs, because affective states can be highly variable (Larson et al., 1980) and perceptions of others or interactions may be context-dependent (e.g., dependent on the type of company; Tsi & Reis, 2009). Therefore, a more precise and accurate way to measure both affect and perceptions of others may be by using momentary assessments.

Loneliness, Mood, and Perception of Social Interactions: Momentary Assessments

Only a few studies have examined relations between trait levels of loneliness and momentary assessments of mood. These studies used the Experience Sampling Method (ESM, Csikszentmihalyi & Larson, 1987), which is used to assess participants’ experiences in their daily living environment. Compared to more conventional methods, this method has two important advantages. Participants do not have to rate their mood states retrospectively, thereby preventing recall bias, and the ecological validity of this method is very high, because participants fill out questionnaires in their natural environment (Myin-Germeys et al., 2009). In earlier ESM studies on both young and old adults, baseline levels of loneliness were related to higher levels of momentary negative affect and lower levels of momentary positive affect (Hawkley, Preacher, & Cacioppo, 2007; Steptoe et al., 2011).

Regarding the relation between loneliness and perceptions of others and interactions with others, an ESM study on young adults found that lonely people perceived their interactions with others more negatively and less positively than non-lonely people do (Hawkley et al., 2003). This pattern was confirmed by a study on older adults (Roock, 2001), in which loneliness was related to more negative and less positive perceptions of daily social interactions.

In sum, these results show that lonely people of different ages experience lower levels of momentary positive affect and higher levels of momentary negative affect and that lonely people perceive their social interactions more negatively and less positively. The first goal of the present study is to replicate these results in adolescents, by examining whether trait levels of loneliness are related to momentary positive and negative affect. Because lonely people had more negative perceptions of their interactions, we also examined whether trait levels of loneliness were related to momentary positive and negative perceptions of the company the adolescents were in.

A Socio-Cognitive Model of Loneliness

What has been lacking in research on loneliness, affect, and perception is an overarching model in which these constructs are brought together. Importantly, Cacioppo and Hawkley (2009) presented a theoretical model of loneliness in which both negative affectivity and negative perceptions of others are included. According to this model, lonely people are characterized by (a) a hypervigilance to social threat and (b) a lower reward experience in response to positive social events or stimuli. Regarding the first characteristic, that is, hypervigilance to social threat, previous studies found that lonely people have greater visual attention for negative social stimuli than for negative non-social stimuli (Cacioppo et al., 2009) and view their daily activities as more threatening than non-lonely people do (Hawkley et al., 2003).

Regarding the second characteristic, that is, lowered reward experience, it was found that the activation in reward areas in the brain (i.e., ventral striatum) in response to pleasant social pictures was lower in lonely compared to non-lonely people (Cacioppo et al., 2009), indicating that lonely people were not rewarded by pleasant social stimuli to the extent that non-lonely people are. These two characteristics may both cause increasingly negative expectations about relationships and social interactions, thereby contributing to a vicious circle of lowered positive and increased negative affect.

In the present study, we aimed to examine these two characteristics in the daily lives of adolescents, by examining relations between perceptions of company and affect, and whether these relations were moderated by loneliness. Because we could not conduct exact assessments of hypervigilance to social threat, we examined general emotional responses to company that is perceived as threatening and judgmental (from now on referred to as responses to perceived social threat), as we investigated the moderating role of loneliness in the relation between negative perceptions of company and positive and negative affect. In addition, we examined general positive emotional responses to company that is perceived as comforting and accepting. (Less pronounced positive responses to such company are from now on referred to as lowered reward response).

Although this approach does not measure cognitive biases directly, it does provide important insights into the subjective experiences of adolescents. In the present chapter, we changed the names of the two characteristics of the socio-cognitive model (i.e., hypervigilance to social threat and hypersensitivity to social threat) into responses to perceived social threat and lowered reward response, to comply with a reviewers’ query.
There are several reasons why it is important to examine these characteristics in early adolescence. First, regarding the responses to perceived social threat, previous research found that, compared to pre-pubertal adolescents (mean age 11.8), mid- to late adolescents (mean age 12.4) showed less brain reactivity to ambiguous threatening stimuli (i.e., fearful faces), whereas they showed more brain reactivity to non-ambiguous threatening stimuli (i.e., angry faces) (Forbes, Phillips, Silk, Ryan, & Dahl, 2011). In addition, higher reactivity to all threatening stimuli was related to higher levels of subjective negative affect and depressive symptoms, indicating that adolescents who are vigilant to social threat also experience more negative emotions in their daily lives.

Second, regarding the lowered reward response, previous research has shown that connections between the dopaminergic system and the prefrontal cortex are strengthened in early adolescence, which makes adolescents increasingly able to respond to more complex and temporally distant rewards, and also form new goals based on these rewards (Davey et al., 2008). Hence, as their social world becomes more complicated, adolescents are also able to strive for complex interpersonal rewards. Davey et al. (2008) hypothesized that when these rewards are not fulfilled, feelings of frustration and disappointment (e.g., loneliness) can lead to suppression of the reward system, thereby making adolescents less susceptible to social rewards. In sum, important changes have been found to occur in early adolescence in responses to perceived social threat and lowered reward response. So it is crucial to examine these characteristics, which are based in large part on studies conducted on young adults, in early adolescents as well.

The Present Study

The first goal of the present study was to examine the relations between loneliness and momentary positive and negative affect, and between loneliness and the momentary positive and negative appraisals of the company adolescents were in (from now on referred to as “positive and negative company”). We expected loneliness to be related to higher levels of momentary negative affect and negative company and to lower levels of momentary positive affect and positive company.

The second goal was to examine whether the presumed response to perceived social threat and the lowered reward response to positive stimuli in lonely people could be found in our momentary data. First, we examined whether loneliness influenced the relations between negative company and negative and positive affect (i.e., response to perceived social threat). We expected lonely adolescents to be more negatively affected by negative company than non-lonely adolescents. Second, we examined whether loneliness affected the relations between positive company and positive and negative affect (i.e., lowered reward response). We expected adolescents who scored high on loneliness to be less positively affected by positive company than adolescents who scored low on loneliness. Finally, as we focused on loneliness in relation to peers, it may be that adolescents high on loneliness experience different levels of affect when they are with peers than adolescents low on loneliness. Therefore, we examined whether the type of company adolescents were in (e.g., friends, family, classmates or others) affected the relations in our models.

Method

Procedure

Adolescents were recruited through secondary schools. Schools were sent information letters in which they were asked to participate in the present study. When the school consented, all adolescents in the second year and their parents were sent a letter in which information about the study was provided. If adolescents agreed to participate, a consent form had to be signed by their parents and an assent form by the adolescents themselves. No exclusion criteria were used. All adolescents with permission from their parents could participate in the study.

The study consisted of two parts: the baseline questionnaire and the momentary assessments. Administration of the baseline questionnaire, in which trait levels of loneliness were measured, took place two to eight weeks before the start of the momentary assessments. Adolescents filled out the questionnaire online, during school hours. Daily data were collected by using the Experience Sampling Method (Myin-Germeys et al., 2009), which is used to assess adolescents’ experiences in their daily living environment. Data collection took place during a 6-day long sampling period, always starting on Fridays. We used smartphones, on which the program MyExperience was installed (Froehlich, Chen, Consolvo, & Harrison, 2007). This is an open source tool designed to collect data in the daily lives of adolescents. Each week, 20 adolescents participated. The smartphones were programmed to emit buzzing signals nine times a day at random time points during 90-minute intervals, after which adolescents filled out the questionnaire. It took around three minutes to complete a questionnaire. When adolescents did not respond within two minutes after the signal, the buzzing signal was emitted again, with a maximum of three reminders. This procedure implied that adolescents who did not respond within eight minutes after the first signal were not able to fill out that questionnaire. In total, participants could fill out 54 momentary assessments (i.e., nine assessments per day, for six days).

One day before the start of the ESM study, adolescents received the smartphone and were individually briefed on how to use the smartphones. They were instructed to turn on the smartphone as soon as they woke up in the morning and to turn it off when they went to sleep at night. In addition, we instructed the adolescents to pause their activity immediately after they received a signal, and fill out the questionnaire on the smartphone. A mobile telephone number was provided, which adolescents could call in case of any problems. Data were stored on the smartphones and a text message was sent to the
principal investigator after each completed questionnaire, making it possible to check whether adolescents complied. When no messages were received within two consecutive hours, adolescents were sent a text message or called to instruct them to attend to their smartphones and fill out the questionnaires. On the final day of the study, adolescents were called to make an appointment for returning the smartphones and filling out a final short questionnaire. In this questionnaire, we checked whether any special, atypical events took place during the sampling period and whether any problems occurred. Adolescents received the full reward of €20 (i.e., about 27 US$) when they completed at least 55% of the momentary assessments.

Participants

Data were collected in four secondary schools. Of the total group of adolescents contacted (N = 933), 32.5% agreed to participate (N = 339). A small number of this group was not able to participate due to illness or organizational problems, and a few students withdrew their consent. Therefore, our final sample consisted of 303 adolescents, aged between 13 and 16 years (M age = 14.19). Of the total sample, 59% were girls and 97.3% were born in The Netherlands. The different types of education were all well represented in the sample: 23.4% of the adolescents attended preparatory secondary school for technical and vocational training, 35.8% attended preparatory secondary school for college, and 40.8% attended preparatory secondary school for university. The present study was approved by the Medical Ethical Committee Arnhem-Nijmegen.

Materials

Baseline measures. Loneliness was measured in the baseline questionnaire with the peer-related subscale of the Louvain Loneliness Scale for Children and Adolescents (LLCSA, Marcoen et al., 1987). This scale consisted of 12 items, which had to be rated on a 4-point scale, ranging from (1) never to (4) often. A sample item was “I think I have fewer friends than others have.” Cronbach’s alpha was .88. Depressive feelings were measured with the Center for Epidemiology-Depression scale (CES-D, Radloff, 1977). This scale had 20 items, rated on a four-point scale (1 = never, 4 = often) Cronbach’s alpha was .90. Finally, social anxiety symptoms were measured with a subscale of the Brief Symptom Inventory (BSI, Derogatis & Melisaratos, 1983). This scale consisted of four items, which were rated on a five-point scale, ranging from (1) not at all to (5) very much. Cronbach alpha was .75. A sample item was “I feel that others are unfriendly or do not like me”.

Momentary assessments. Momentary assessments were obtained by using the Experience Sampling Method. Positive and negative affect were measured by five items each. These items were selected from items used in other ESM studies (e.g., Peeters, Berkof, Delespaul, & Rottenberg, 2006; Wichers et al., 2007). Positive affect was measured by the items joyful, satisfied, happy, energetic, and cheerful. Negative affect was measured by the items insecure, anxious, worried, low, and guilty. Adolescents had to rate to what extent they experienced the described emotion on a 7-point scale ranging from (1) not at all to (7) very much. Cronbach’s alpha across all momentary assessments (i.e., 10,865 assessments) was .84 for positive affect and .76 for negative affect.

When adolescents were not alone, positive and negative company were measured. Positive company consisted of the items “I feel accepted by this company” and “I feel comfortable in this company” (r = .60). Negative company consisted of the items “I feel threatened by this company” and “I feel judged by this company” (r = .37). In addition, adolescents reported in an open-ended question with whom they were. These responses were coded into family (e.g., parents, siblings), friends, classmates, or others (e.g., teachers, team mates). To calculate the intra-rater reliability, 10% of the total number of assessments in company (N = 676) were randomly selected and coded by a different rater. This resulted in a kappa of .97 (p < .001), indicating good interrater reliability.

Results

Momentary Data Preparation

The total dataset consisted of 10,865 momentary assessments. The average number of completed momentary assessments per adolescent was 37 out of a maximum of 54 (SD = 11.12). Of the participating adolescents, 17 adolescents (i.e., 5.61%) had less than 18 completed momentary assessments (i.e., one-third of the maximum number of assessments), which was the minimum to be included in the analyses. Because positive and negative company were only measured when adolescents were not alone, only the observations in which adolescents were with others were included in the analyses. Some of the adolescents had very few observations in which they were with others (range of the number of observations in company was 2 to 51, M = 23.29, SD = 8.94). Therefore, we excluded adolescents who had less than 10 momentary assessments in which they were with others (i.e., 2.64%, N = 8). This resulted in a dataset with 6,583 momentary assessments in 278 adolescents. We checked whether adolescents who were not included in the analyses (N = 25) differed from adolescents who were included (N = 278) on demographic characteristics and baseline levels of loneliness and depressive feelings. No significant differences were found (p > .05). In addition, we examined correlations between compliance (i.e., number of assessments) and all model variables. No correlations were found between compliance and mean levels of positive and negative company. We did find small correlations between compliance and baseline levels of loneliness (r = .13, p < .05), and with mean levels of positive affect (r = .12, p < .05) and negative affect (r = -.12, p < .05). These correlations indicate that adolescents with high compliance rates had higher levels of baseline loneliness, higher levels of mean positive affect, and lower levels of mean negative affect, compared to adolescents with low compliance rates.
Descriptive Statistics

Descriptive statistics and correlations for the model variables, with scores averaged across assessments for all Experience Sampling measures, can be found in Table 1. As can be seen, average levels of loneliness were relatively low, considering the potential range (i.e., 12 to 48). However, this level was comparable to average levels found in other community samples (e.g., van Roekel, Goossens, Scholte, Engels, & Verhagen, 2011). Mean levels for both positive company and positive affect were higher and more variable than was the case for negative company and negative affect (r [277] = 75.58, p < .001, r [277] = 61.01, p < .001, respectively). As expected, baseline levels of loneliness were related to higher levels of negative company and negative affect, and lower levels of positive company and positive affect. Low but significant correlations were found between sex and most model variables, in that girls had lower levels of positive affect, and higher levels of loneliness, negative affect, and negative company. Depressive feelings and social anxiety symptoms were significantly related to the different momentary assessments of affect and perceptions of company. Therefore, we decided to control for sex, depressive feelings, and social anxiety symptoms in all further analyses. No correlations were found between age and the other variables. Regarding the type of company, adolescents spent most time with classmates (42% of the total number of assessments of company) and family (36%), and less time with friends (18%) and others (4%).

Model Results

In the present study, the repeated momentary assessments (Level 1) were nested within individuals (Level 2). Therefore, multilevel linear regression analyses were conducted in Mplus (Muthén & Muthén, 1998-2007). An advantage of this approach is that it handles missing data, in that it does not require adolescents to have data at each assessment. In addition, in this approach it is possible to examine Level 1 predictors as random coefficients, making it possible to examine whether the relations between the Level 1 variables vary across adolescents (Hox, 2010). For example, by using this approach, we can examine whether the relation between the Level 1 variables negative company and affect varies across adolescents, by including negative company as a random predictor in the model. When this coefficient is significant, this implies that the relation between negative company and affect differs between adolescents, and can therefore be predicted by individual characteristics (i.e., Level 2 predictors).

First, unconditional models were tested, including only a constant and a dependent variable. The intra-class correlation was .38 for negative affect (NA), .37 for positive affect (PA), .34 for negative company (NC), and .28 for positive company (PC), indicating that around 38% of the variation in negative and positive affect and, respectively, 28 to 34% of the variation in positive and negative company occurred at the individual level. The results showed that all dependent variables had significant amounts of variance at Levels 1 and 2 (Level 1 variance NA = .27, Level 2 variance NA = .17, Level 1 variance PA = .73, Level 2 variance PA = .44; Level 1 variance NC = .49, Level 2 variance NC = .25; Level 1 variance PC = .77, Level 2 variance PC = .30). This indicates that a significant amount of variance in the dependent variables occurred at the assessment level (i.e., Level 1), as well as the individual level (i.e., Level 2).

Second, we examined whether baseline levels of loneliness were related to positive and negative company. Because positive and negative company are likely to be correlated, we examined this in a multivariate multilevel model. In such a model, multiple outcome measures (i.e., positive and negative company) are included simultaneously, allowing the outcome measures to be correlated (Hox, 2010). Loneliness was negatively related to positive company (B = -13, SE = .04, p < .01), and positively related to negative company (B = 11, SE = .04, p < .01), indicating that higher levels of baseline loneliness were related to lower levels of momentary positive company and higher levels of momentary negative company.

Third, we examined whether baseline levels of loneliness were related to positive and negative affect in a multivariate multilevel model. We controlled for levels of positive and negative affect at the previous assessment, sex, depressive feelings, and social anxiety symptoms. Loneliness was negatively related to positive affect (B = -01, SE = .05, p < .05), whereas the relation with negative affect was not significant (B = .01, SE = .04, p > .05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>M</th>
<th>SD</th>
<th>N</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
<th>8.</th>
<th>9.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Baseline loneliness</td>
<td>17.68</td>
<td>5.39</td>
<td>273</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Age</td>
<td>14.19</td>
<td>0.55</td>
<td>277</td>
<td>-08</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Sex*</td>
<td>0.59</td>
<td>0.49</td>
<td>278</td>
<td>18**</td>
<td>-11</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Depressive feelings</td>
<td>28.70</td>
<td>7.79</td>
<td>272</td>
<td>53**</td>
<td>-10</td>
<td>21**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Social anxiety</td>
<td>1.92</td>
<td>0.72</td>
<td>272</td>
<td>63**</td>
<td>-18**</td>
<td>25**</td>
<td>63**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Negative company</td>
<td>1.52</td>
<td>0.53</td>
<td>278</td>
<td>33**</td>
<td>-04</td>
<td>06</td>
<td>33**</td>
<td>30**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Positive company</td>
<td>6.16</td>
<td>0.60</td>
<td>278</td>
<td>-39**</td>
<td>05</td>
<td>03</td>
<td>-40**</td>
<td>-39**</td>
<td>-66**</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Negative affect</td>
<td>1.45</td>
<td>0.45</td>
<td>278</td>
<td>28**</td>
<td>02</td>
<td>18**</td>
<td>45**</td>
<td>36**</td>
<td>60**</td>
<td>-54**</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9. Positive affect</td>
<td>5.17</td>
<td>0.73</td>
<td>278</td>
<td>-31**</td>
<td>-02</td>
<td>05</td>
<td>-36**</td>
<td>-30**</td>
<td>-28**</td>
<td>53**</td>
<td>-49</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: *0 = boy; 1 = girl.
* p < .05. ** p < .01.
Depressive feelings were significantly related to both positive ($\beta = -.18, SE = .05, p < .001$) and negative affect ($\beta = .16, SE = .04, p < .001$). Fourth, to test the response to perceived social threat, we examined whether loneliness moderated the relation between negative company and positive and negative affect. Regression coefficients and variance components can be found in Table 2.

First, a baseline model was estimated in which we included only the control variables. Results showed that depressive feelings were related to positive and negative affect, whereas no relation was found between social anxiety symptoms and positive and negative affect. In the next model, we tested whether negative company was related to both positive and negative affect. Negative company was included in the model as a random effect, because we expected the relation between negative company and affect to differ between adolescents. As can be seen in Table 2, negative company was positively related to negative affect, and negatively related to positive affect, indicating that higher levels of negative company are accompanied by higher levels of negative affect and lower levels of positive affect.

Next, the Level-2 predictor loneliness and the cross-level interaction between negative company and loneliness were added to the model. Results showed that the interaction was significantly related to both negative and positive affect. As can be seen in Figure 1, adolescents with the highest levels of loneliness in general had higher levels of negative affect. In addition, compared to adolescents low in loneliness, adolescents with high levels of loneliness showed a steeper increase in negative affect when they were in negative company. Figure 2 shows that adolescents with higher levels of loneliness had lower levels of positive affect, and their positive affect decreased more when they were in negative company, compared to adolescents with low levels of loneliness.

Fifth, we tested whether the relation between positive company and positive and negative affect was moderated by loneliness. To do this, we first examined whether positive company was related to negative and positive affect. Regression coefficients and variance components for this model are shown in Table 3. Positive company was included in the model as a random effect, because we expected the relation between positive company and affect to differ between adolescents. Results showed that positive company was negatively related to negative affect and positively related to positive affect. High levels of positive company were associated with low levels of negative affect and high levels of positive affect. To examine whether loneliness moderated this relation, loneliness and the cross-level interaction between loneliness and positive company were added to the model.

As can be seen in Table 3, the cross-level interaction was significant for negative affect. Figure 3 shows that adolescents with high levels of loneliness had the highest level of negative affect, and decreased faster in negative affect when they were in positive company, compared to adolescents with low levels of loneliness. In addition, the cross-level interaction was at trend-level for positive affect ($p = .06$). Although not

### Table 2 Multivariate Multilevel Models for Relations Between Negative Company, Loneliness, and Negative and Positive Affect

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative affect</th>
<th>Positive affect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Level-1: random</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.46***</td>
<td>1.44***</td>
</tr>
<tr>
<td>Affect t-1</td>
<td>22 (0.02)**</td>
<td>17 (0.02)**</td>
</tr>
<tr>
<td>Sex</td>
<td>.07 (0.05)</td>
<td>.07 (0.05)</td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>.16 (0.04)**</td>
<td>.16 (0.04)**</td>
</tr>
<tr>
<td>Social anxiety</td>
<td>.06 (0.04)</td>
<td>.05 (0.03)</td>
</tr>
<tr>
<td>Negative company</td>
<td>1.7 (0.02)**</td>
<td>1.7 (0.02)**</td>
</tr>
<tr>
<td>Loneliness</td>
<td>0.2 (0.04)</td>
<td>0.2 (0.04)</td>
</tr>
<tr>
<td>Loneliness x Company</td>
<td>0.03 (0.01)*</td>
<td>0.03 (0.01)*</td>
</tr>
<tr>
<td>Level-1 variance</td>
<td>.29 (0.02)**</td>
<td>.24 (0.02)**</td>
</tr>
<tr>
<td>Level-2 intercept variance</td>
<td>.16 (0.02)**</td>
<td>.14 (0.02)**</td>
</tr>
<tr>
<td>Level-2 slope variance</td>
<td>.03 (0.01)**</td>
<td>.03 (0.01)**</td>
</tr>
</tbody>
</table>

Note: All observation-level variables were group-mean centered, and all person-level variables were grand-mean centered. † $p < .07$. * $p < .05$. ** $p < .01$. *** $p < .001$.

significant, this finding indicated that adolescents high in loneliness had a steeper increase in positive affect when they were in positive company.
levels of negative affect when they were with classmates ($B = .14, SE = .03, p < .001$), and lower levels of positive affect when they were with family ($B = -.21, SE = .04, p < .001$) or classmates ($B = -.36, SE = .04, p < .001$). No significant differences in affect were found between being with friends and being with others ($B = .05, SE = .06, p > .05$ for negative affect; $B = -.14, SE = .07, p > .05$ for positive affect). Compared to classmates, adolescents...
experienced lower levels of negative affect with family ($B = -1.1, SE = .02, p < .001$) and friends ($B = -1.3, SE = .03, p < .001$), and higher levels of positive affect with family ($B = .15, SE = .03, p < .001$), friends ($B = .35, SE = .04, p < .001$), and others ($B = .22, SE = .07, p < .01$). No differences in negative affect were found between being with classmates and with others ($B = -.08, SE = .05, p > .05$).

Second, we examined whether loneliness moderated these relations by adding the cross-level interactions between the dummy variables for type of company and loneliness to the model, again using friends and classmates as the reference groups. For friends as reference group, we found no significant cross-level interactions ($Bs$ ranging from -.03 to .06, $ps$ ranging from .11 to .68; a full description of analyses is available from the first author), which indicated that adolescents high on loneliness did not feel better or worse than adolescents low on loneliness in situations with friends compared to the other types of company. For classmates, we did find a significant crosslevel interaction for being with family ($B = -.07, SE = .03, p < .05$). Compared to adolescents low on loneliness, adolescents with higher levels of loneliness experienced lower levels of negative affect when they were with family, compared to classmates. None of the other cross-level interactions were significant.

Third, we examined whether the cross-level interaction between perceptions of company and loneliness on affect differed for type of company. In other words, we aimed to examine whether adolescents high on loneliness would be more positively or negatively affected by their perceptions of a specific type of company. To do this, we entered three-way interactions between perceptions of company (positive and negative), type of company, and loneliness, on positive and negative affect. No significant three-way interactions were found.

**Discussion**

The first goal of the present study was to examine whether lonely adolescents experienced higher levels of momentary negative affect and lower levels of momentary positive affect, and whether they perceived their company more negatively and less positively. Second, we tested responses to perceived social threat and lowered reward responses in lonely adolescents, by examining whether loneliness moderated the relations between negative company and negative and positive affect (i.e., response to perceived social threat) and whether loneliness moderated the relations between positive company and negative and positive affect (i.e., lowered reward experience).

**Loneliness, Affect, and Perceived Company**

Our results showed that baseline levels of loneliness were related to higher levels of negative affect and lower levels of positive affect, as expected. We also found that loneliness was related to how people perceive the company they are in. Adolescents who were more lonely perceived the company they were in as less positive and more negative than adolescents who were less lonely. These findings were in line with our expectations and previous research using ESM, in which lonely people perceived their interactions with others more negatively (Hawkley et al., 2007).

**The Socio-Cognitive Model of Loneliness**

According to the socio-cognitive model of loneliness, lonely people are characterized by hypervigilance to social threat and lowered reward response to positive stimuli (Cacioppo & Hawkley, 2009). In the present study, we examined responses to perceived social threat, which may be a consequence of hypervigilance to threat. Regarding the responses to perceived social threat, our findings were in line with our expectations. Adolescents who scored high on loneliness were more negatively affected by negative company than adolescents who scored low on loneliness, which indicates that adolescents high in loneliness indeed experienced greater negative responses to perceived social threat. Further research is necessary to examine how these heightened responses to perceived social threat are related to the hypervigilance to social threat as it is described in the socio-cognitive model. A possible way to examine this may be to combine experiments with momentary assessments. For example, adolescents could be exposed to social threatening situations or stimuli in an experimental setting, by which their vigilance to social threat is examined. Next, they could participate in an ESM study, in which the affective responses to perceived social threat are tested. In this way, it is possible to examine whether adolescents who score high on vigilance to social threat also experience more negative responses to perceived social threat.

The finding regarding the heightened responses to social threat alone could lead us to conclude that lonely people perceive their world negatively, and that this negative
perception in turn is related to stronger negative feelings and less positive feelings, thereby resulting in a negative vicious circle. However, the findings regarding the lowered reward response to positive stimuli provide evidence for a more optimistic view. In contrast to our expectations and the loneliness model (Cacioppo & Hawkley, 2009), we found that lonely people actually seemed to benefit from positive company, because their levels of negative affect decreased more when they were in positive company than the levels of negative affect in non-lonely people. This finding indicates that adolescents with higher levels of loneliness are rewarded by positive company, and even more so than adolescents with lower levels of loneliness.

There are several potential explanations for this particular finding. First of all, our findings may differ from those of Cacioppo et al. (2009) because the fMRI study on which this characteristic is based examined this aspect of socio-cognitive functioning by looking at brain activation in reward areas in response to positive social stimuli (Cacioppo et al., 2009). Therefore, the difference in results may be due to the type of measure used (i.e., fMRI data vs. self-reports). In the present study, lonely people who rated their company as pleasant had stronger decreases in their negative affect than non-lonely people who rated their company as pleasant. In the fMRI study by Cacioppo et al. (2009), participants viewed social pictures that did not represent social interactions or social relationships (e.g., a pleasant social picture depicted a man and a dog running). Therefore, it would be interesting to examine in future research whether lonely people show more or less activation in reward brain areas in response to pictures of people that they perceive as positive versus negative company. It could be that lonely people are less rewarded by general social stimuli, but are rewarded when they view pictures that represent meaningful situations.

Second, the loneliness model is based on people who are chronically lonely, or at least have high levels of loneliness (e.g., Hawkley et al., 2003). As loneliness scores were relatively low in our sample, our results may not entirely concur with the loneliness model because the adolescents with the highest scores on loneliness still scored relatively low compared to the potential range of the scale. It could be that adolescents who feel lonely but who do not have very severe levels of loneliness do benefit from being in positive company, whereas results may be different for adolescents with severe levels of loneliness. In future research, it may be important to examine whether people with low versus high levels of loneliness are rewarded by positive company. It is important to note, however, that our study provides valuable information on how these characteristics are present in a normative sample of adolescents.

Previous research has shown that responses to perceived social threat and lowered reward responses also play a role in depression (Davey et al., 2008) and (social) anxiety (Bar-Haim, Lamy, Perfgamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Therefore, we controlled for depressive feelings and social anxiety symptoms. Important to note is that we found a significant relation between loneliness and negative affect, but this relation was non-significant when we included the covariates. This finding indicates that adolescents who feel more lonely, also are likely to be more depressed, and that these depressive feelings account for the variation in negative affect. Because of this finding, and the fact that the responses to perceived social threat and the lowered reward responses seem to play a role in loneliness, depression, and anxiety, it is important to examine in future research what the underlying mechanism is of the relations between these characteristics and internalizing problems. Further research should examine relations between the characteristics and internalizing problems longitudinally.

In our additional analyses, we checked whether the results were affected by the type of company adolescents were in. We found that compared to being with friends, adolescents experienced higher levels of negative affect when they were with classmates, and lower levels of positive affect when they were with family. These findings are in line with previous studies (Larson, 1983; Silk et al., 2011), which found that adolescents experienced more positive affect (Larson, 1983) or a greater positive-negative affect ratio (Silk et al., 2011) when they were with friends or peers, compared to when they were with family. In addition, we found that, compared to being with classmates, adolescents experienced lower levels of negative affect with family and friends, and higher levels of positive affect in situations with family, friends, or others. These findings indicate that being with classmates, which is likely to be during school hours, is a relatively negative experience for adolescents. This could be a cause for concern, as adolescents spend more time in company with classmates at school than in any other company.

Next, we examined whether loneliness moderated the relations between type of company and affect. We found no differences between being with friends and the other types of company, which indicated that adolescents low or high in loneliness did not feel better or worse in a specific type of company. However, when we compared classmates with the other types of company, we found that adolescents high in loneliness had significantly higher levels of negative affect when they were with classmates, compared to situations with family. For adolescents high in loneliness, being with classmates may be a particularly negative situation, as there are more opportunities for rejection in those situations. Being with family on the other hand, may be a relatively safe situation, as loneliness generally arises from not feeling connected with peers, not with family members.

Finally, we tested whether the interaction between perceptions of company and loneliness differed for the different types of company. No significant three-way interactions were found, indicating that adolescents high on loneliness are more susceptible for positive and negative company, independent of who that company is.

**Strengths and Limitations**

The most important strength of our study is that we used the Experience Sampling Method, making it possible to examine affect and appraisals of company in real time in real life. Furthermore, by using multiple measurement points, the random error variance is
reduced (Myin-Germeys et al., 2009). However, some limitations have to be mentioned. First, participation in this study was voluntary and adolescents had to provide active consent. Therefore, we may have selected a relatively healthy sample, because adolescents with higher levels of problem behavior (e.g., loneliness) may not be inclined to participate in a time-consuming ESM study. As we do not have information on the adolescents who declined to participate, we cannot compare loneliness levels between the two groups. However, the mean levels of baseline loneliness in our sample are comparable to other community samples. Therefore, we might conclude that we did not select a sample with extremely low levels of loneliness.

Second, all variables were measured by means of self-reports, including the measure of company that we used as a threat measure (i.e., negative company) or as a reward measure (i.e., positive company). It may be that more objective measures of threat or reward, such as social and non-social stimuli, or positive and negative facial expressions, have different effects. Future studies could try to combine fMRI measures of threat and reward experiences with daily life measures (see, e.g., Forbes et al., 2011). Related to this, because we measured the subjective experiences of adolescents, we do not know whether the actual social situations of adolescents high in loneliness are objectively less positive than the social situations of adolescents low in loneliness. Surprisingly, very little research has examined the actual social relations of lonely people. Studies examining actual social acceptance in children and adolescents did find that low peer-accepted children and adolescents were more lonely than high accepted children and adolescents (Kingery, Erdley, & Marshall, 2011; Parker & Asher, 1993). It is also important to note that research in children has shown that not all low peer-accepted children are lonely, and that not all lonely children are low peer-accepted (Qualter & Munn, 2002). Within the context of our study design, in which actual social relations were not measured, it is difficult to find out whether adolescents high on loneliness perceive their environment more negatively, or whether their environment actually is more negative than for adolescents low on loneliness. Further research is needed to examine these relations in more detail.

The third limitation concerns our sample, which consisted of healthy, Caucasian adolescents. Although our study provides important results on the relations between loneliness, mood, and perception in this normative group, future studies could consider including adolescents with more pronounced levels of loneliness. In such a way, it is possible to examine whether the relations between company and affect we found are also present in adolescents with more severe levels of loneliness. Another limitation may be that adolescents who agree to participate typically have a higher socioeconomic status (based on parental education) than adolescents who do not agree to participate (Larson, Raffaelli, Richards, Ham, & Jewell, 1990). Unfortunately, we did not have information on the adolescents who did not agree to participate. However, our sample did represent adolescents from lower, middle, and higher educational levels, which could indicate that overrepresentation of higher socio-economic strata was not a problem in our sample.

Finally, an important problem in ESM studies is that the data collection is unsupervised (Schneiders et al., 2007), making it hard to check whether participants comply with the signal and fill out the questionnaires on time. However, because we used smartphones, the time at which the adolescents filled out the questionnaire was automatically registered. In addition, because we received text messages when a questionnaire was filled out, we were able to check whether adolescents complied. As mentioned in the Method section, the compliance during data collection was moderate (with only 17 adolescents who did not have the minimum of 18 out of 54 momentary assessments).

Conclusions

The present study was the first to examine relations between loneliness, affect, and perceptions of others in adolescents, using the Experience Sampling Method. Our results contribute to a further understanding of how trait levels of loneliness are related to affect and perceptions of others in daily life. Our main finding was that adolescents high in loneliness are more negatively affected by negative company than adolescents low in loneliness which was in line with our expectations. On the other hand, we found that adolescents high in loneliness were also more positively affected by positive company than adolescents low in loneliness, in that their levels of negative affect decreased more when they were in positive company. This finding was not in line with the socio-cognitive model of loneliness, which states that lonely people are not rewarded by positive social stimuli (Cacioppo & Hawkley, 2009). Future studies could combine several methodological approaches (e.g., fMRI studies and momentary assessments) to further examine this socio-cognitive model of loneliness.
Chapter 5
Loneliness in the daily lives of late adolescents: Testing a socio-cognitive model

Submitted as:
van Roekel, E., Ha, T., Verhagen, M., Scholte, R. H. J., & Engels, R. C. M. E. Loneliness in the daily lives of late adolescents: Testing a socio-cognitive model.
CHAPTER 5

LONELINESS IN THE DAILY LIVES OF LATE ADOLESCENTS

Introduction

People have a rudimentary need to belong, which affects our behavior, cognitions, and emotions (Baumeister & Leary, 1995). When this need to belong is thwarted, feelings of loneliness can arise. Loneliness is defined as the negative emotional response to an experienced discrepancy between the desired and actual social relationships (Perlman & Peplau, 1981). Chronic levels of loneliness can have severe health consequences, such as altered cardiovascular regulation (e.g., Cacioppo et al., 2002), less salubrious sleep (e.g., Hawkley et al., 2010; Kurina et al., 2011), and poorer immune responses (Pressman et al., 2005). Importantly, higher levels of loneliness are found to increase chances of mortality by as much as 50% (Holt-Lunstad et al., 2010). These findings highlight the significance of social connections and loneliness for wellbeing and even survival.

The transition from high school to college seems to be a challenging period in the social lives of late-adolescents, as they often leave their parents’ home and have to establish new social relationships, while maintaining the existing relationships. The difficulties that late adolescents experience with these transitions have been related to decreases in emotional wellbeing, such as higher levels of depressive feelings and particularly increased feelings of loneliness (Stroebe, van Vliet, Hewstone, & Willis, 2002).

Given these important social changes during late-adolescence, the present study will focus on examining loneliness in first-year college students. In order to prevent or reduce feelings of loneliness, it is important to examine factors that can predict or maintain feelings of loneliness. More specifically, we examined two core characteristics; (a) hyper-sensitivity to social threat and (b) hyposensitivity to social reward, in the daily lives of late adolescents.

Several studies have confirmed that hypersensitivity to social threat plays a role in the development and continuation of loneliness. Studies in college students found that lonely people have greater visual attention to negative social stimuli than negative non-social stimuli (Cacioppo et al., 2009), indicating that lonely people pay attention particularly to social threats, relative to nonsocial threats. Results from both an observational study...
indicate that the findings of Van Roekel et al. (2013), that lonely adolescents are more susceptible to social reward than older samples, which could explain their hyposensitivity to reward, especially in social contexts (Davey et al., 2008). Therefore, early adolescents may be more sensitive to social reward than older samples, as they are more motivated to interact with others and receive social attention (Davey et al., 2008). A possible explanation for this difference is that early adolescents are more motivated to interact with others and receive social attention (Davey et al., 2008).

Regarding hyposensitivity to social reward, an fMRI study in college students showed that lonely people have lower responses in brain reward areas (i.e., ventral striatum) than non-lonely people in response to positive social stimuli (Cacioppo et al., 2009). In addition, lonely people have decreased activation in reward areas in the brain when viewing positive social stimuli, compared to viewing positive non-social stimuli, indicating that the hyposensitivity for reward in lonely people is specific to social stimuli (Hawkley et al., 2003; Hawkley et al., 2007). Interestingly, an ESM study in adolescents found that adolescents high in loneliness had less positive perceptions of others, but in contrast showed greater reductions in negative affect when they perceived their company more positively, as they experienced lower levels of negative affect in positive company than adolescents low in loneliness (van Roekel et al., 2013).

These findings in adolescents are in contrast with the socio-cognitive model of loneliness, in that they indicate that adolescents high in loneliness benefited more, and not less, from positive social contexts than adolescents low in loneliness. A possible explanation for this difference in findings is that the ESM study among adolescents (van Roekel et al., 2013) used subjective perceptions of company as a measure of social reward, whereas the fMRI study that found support for hyposensitivity to reward (Cacioppo et al., 2009) used objective positive social stimuli. In addition, the difference in findings may be due to the age of both samples. It could be that early adolescents are more sensitive to positive environments than late adolescents because of the neurobiological developments in early adolescence, which make adolescents increasingly able to respond to more complex and temporally distant (social) rewards (Davey et al., 2008). Therefore, early adolescents may be more susceptible to social reward than older samples, which could indicate that the findings of Van Roekel et al. (2013) that lonely adolescents are more susceptible to positive environments, are specific for early adolescents. It is hence important to examine whether the same pattern of findings can be replicated in an older adolescent sample.

The Current Study

Therefore, the aim of the present study is to examine the two characteristics of the socio-cognitive model in a predominantly female sample of college students by using the ESM. The main advantages of this method are that the ecological validity is high and recall bias is low, as participants fill out questionnaires while living their normal lives (Myin-Germeys et al., 2009). Thus, results can contribute to a further understanding of how lonely college students experience their daily lives. First, hyposensitivity to social threat was examined by investigating whether the associations between negative perceptions of company and positive and negative affect were moderated by loneliness. Second, hyposensitivity to social reward was examined by testing whether the relations between positive perceptions of company and positive and negative affect were moderated by loneliness.

We hypothesized that students high in loneliness would perceive their company as less positive and more negative and that they would experience higher levels of negative affect and lower levels of positive affect. Regarding hyposensitivity to social threat, we expected students high in loneliness to be more affected by negative perceptions of company, in that they would experience higher levels of negative affect and lower levels of positive affect when they perceived their company negatively. Regarding hyposensitivity to social reward, we did not have a specific hypothesis, as studies in college students showed results in favor of hyposensitivity (Cacioppo et al., 2009), whereas an ESM study in adolescents showed results in contrast with hyposensitivity (van Roekel et al., 2013). Because previous research has shown that both hyposensitivity to threat and hyposensitivity to reward play a role in depression as well (e.g., Davey et al., 2008), we controlled for depressive symptoms in all analyses.

Method

Participants

The sample of this study consisted of 219 first year Psychology and Educational Science college students (91% female), with a mean age of 19.60 (SD = 1.46). Of this sample, 76% was of Dutch origin, 21% was born in Germany, and 3% was born in another country. Most students left their parents’ home for college (65% versus 45% living with their parents), typically in student homes, and about half of them were in a relationship at baseline (49%). Almost all students were in their first year of college (96%).
Procedure

All participants were recruited via an Internet sign-up program of the Behavioural Science Institute of the Radboud University Nijmegen, the Netherlands. Attention to this study was elicited by given short introductory talks during several general first and second year classes. Students were informed that they could voluntarily participate by signing up to the Internet program. Participants were required to have a smartphone, as the ESM questionnaires were to be filled out on their smartphone.

The study consisted of three parts, which all took place in November – December of the first year of college. First, participants filled out an online baseline questionnaire, in which questions about demographic characteristics and certain traits were included, e.g. trait loneliness and depressive feelings. Second, one week after administration of the baseline questionnaire, participants were invited to an introduction to the ESM study, which took place in the BSI lab before the start of the momentary assessments. Instructions were given in groups of 4 participants and every item of the daily assessment was reviewed. Participants were instructed to create a new Gmail email address for the present study and to install the Gmail app on their smartphone. This app was programmed to emit a signal whenever participants received a new email on their study email address. Participants were instructed to pause their activity when they received a new email and immediately fill out the questionnaire. We stressed the importance of filling out as many assessments as possible, but also acknowledged that there could be circumstances in which filling out the questionnaires would be impossible, such as during tests, doctor appointments, exercise and so on. We explained that their data were treated anonymously and we notified participants that it was possible to drop out of the study at any moment. In this case they received credits until the point that they quit the study. Participants were provided with an email address for any questions and problems during the momentary assessments. The research team answered these emails daily. As we used an online survey program, we were able to check whether participants filled out the questionnaires, and whether they filled them out on time (i.e., within 20 minutes after the signal). When participants did not fill out enough questionnaires, they were emailed and instructed to attend to the signals on their smartphone. Consent forms were signed at the end of the instruction.

Third, the ESM data collection started one or two days after the instruction. The sampling period consisted of eleven days, with five questionnaires per day, at random time points between 10AM and 11PM on weekdays and between 11AM and 11PM on weekend days (resulting in 55 measurements in total). We used the program Malichimp to send emails to participants on previously determined semi-random time points (i.e., time points were randomly chosen with an average time between time points of 160 minutes). In these emails, a link was provided to an online questionnaire. It took 3-5 minutes to fill out the online questionnaire. Participants received twelve course credits (for educational requirements) when they completed all parts of the study. The Ethical Committee of the

Faculty of Social Sciences, Radboud University Nijmegen, approved the protocols and consent procedures for the present study.

Measures

Baseline loneliness. Trait levels of loneliness were measured in the baseline questionnaire by the University of California, Los Angeles Loneliness scale (UCLA; Russell et al., 1980). This scale consisted of 20 statements and participants were asked to indicate how often they felt the way described in each statement on a four-point scale, ranging from never to always. A sample item is “I lack companionship.” Cronbach’s alpha was .92.

Baseline depressive symptoms. Depressive symptoms were measured in the baseline questionnaire by the Center for Epidemiology-Depression scale (Ces-D; Radloff, 1977), which consisted of 20 items. Participants rate on a four-point scale, ranging from seldom to most of the time or always, to what extent they experienced symptoms of depression in the week prior to assessment. To avoid conceptual overlap with the loneliness measure, we dropped the item ‘I feel lonely’ from the scale, resulting in a 19-item measure with an alpha of .86.

State positive and negative affect. Positive and negative affect were measured during the ESM period by five items each, based on previous ESM research (van Roekel et al., 2013). Positive affect was measured by the items joyful, satisfied, happy, energetic, and cheerful. Negative affect was measured by the items insecure, anxious, worried, low, and guilty. Participants had to rate on a seven-point scale (ranging from not at all to very much) to what extent they experienced those emotions at the moment before the signal. We calculated the reliability of these measures per measurement separately, and averaged these scores to obtain an overall reliability estimate, which resulted in a Cronbach’s alpha of .89 for positive affect and .80 for negative affect.

State perceptions of company. When adolescents were with others, we measured positive and negative perceptions of company. For positive perceptions of company, we used the items “I feel accepted by this company” and “I feel comfortable in this company” (r = .66, p < .001). For negative perceptions of company, the items “I feel threatened by this company” and “I feel judged by this company” were used (r = .34, p < .001).

Momentary Data Preparation

The average number of assessments filled out within the time frame of 20 minutes after the signal, was 35.85 (SD = 9.18). In order to be included in the analyses, participants had to complete at least one-third of the total number of assessments (i.e., 18 out of 55). Based on this criterion, eight participants were removed from the analyses. Further, as our main variables of interest were measured only when adolescents were in company, we excluded those participants who had less than 11 assessments in company (N=12), which resulted in a final sample of 199 participants with in total 3888 momentary assessments. We checked whether the participants excluded from analyses (N=20) differed from those
included in the analyses (N=199) on demographic characteristics (i.e., age, sex) and study variables (i.e., trait loneliness, trait depressive symptoms, mean levels of positive and negative affect and mean levels of positive and negative company). Significant differences were found between participants and drop-outs in aggregated levels of positive affect (t [217] = 2.83, p < .01) and levels of positive company (t [217] = 2.40, p < .05), in that participants had slightly higher levels of positive affect than drop-outs (M [SD] = 4.84 [0.79] for participants, M [SD] = 4.31 [0.94] for drop-outs) and higher levels of positive company than drop-outs (M [SD] = 6.06 [0.61] for participants, M [SD] = 5.71 [0.76] for drop-outs). No significant differences were found for the other variables (p > .05).

Strategy of Analyses
We calculated descriptive statistics for our model variables. For the momentary assessments, scores were aggregated to represent a mean score calculated over all assessments. Because our momentary assessments (Level 1) were nested within individuals (Level 2), we conducted multilevel linear regression analyses in Mplus (Muthén & Muthén, 1998-2007). The main advantage of multilevel analyses is that it can handle missing data, in that it does not require adolescents to have data at each assessment. In addition, in this approach it is possible to examine Level 1 predictors as random coefficients, making it possible to examine whether the relations between the Level 1 variables vary across adolescents (Hox, 2010). In our study, this implied that we could enter the Level 1 predictors positive and negative company as random coefficients, and could examine whether the relations between these Level 1 variables differed for adolescents with different levels of trait loneliness.

First, we tested an unconditional model without predictors, to examine how much of the variance in positive and negative affect could be explained by Level 2 predictors. As positive and negative affect were likely to be correlated, we conducted multivariate multilevel analyses. In such a model, multiple outcome measures (i.e., positive and negative affect) are included simultaneously, allowing the outcome measures to be correlated (Hox, 2010). Second, we examined whether baseline levels of loneliness were related to positive and negative perceptions of company, by using positive and negative company as the dependent (Level 1) variables, and baseline loneliness as the Level 2 predictor. Third, we included baseline levels of loneliness as the Level 2 predictor for positive and negative affect. Fourth, to test hypersensitivity to social threat, we first examined relations between negative company and affect, while controlling for affect at the previous assessment. As we aimed to examine whether baseline levels of loneliness moderated these relations, negative company was added as a random coefficient in the model, making it possible to examine cross-level interactions. Hence, in the next model, baseline loneliness was added as a predictor in the model and the cross-level interaction between loneliness and negative company was examined. Fifth and finally, to test hyposensitivity to social reward, we first entered positive company as a random predictor in the model. In the next model, a cross-level interaction between baseline loneliness and positive company was added to examine whether loneliness moderated the relation between positive company and affect.

Results

Descriptive Statistics
Means, standard deviations, and correlations between study variables are presented in Table 1. Age of participants showed small significant correlations with depressive symptoms and negative company, in that older participants had more depressive symptoms and higher levels of negative company. Age was not related to positive or negative affect, and no correlations were found between sex and any of the model variables. All other model variables were significantly related to each other, higher levels of baseline loneliness and depressive symptoms were related to higher levels of negative affect and negative company, and to lower levels of positive affect and positive company.

Because about half of the sample was involved in a romantic relationship, we checked whether having a relationship or not affected the study variables. No significant mean differences were found between students with and without a romantic relationship for loneliness, depressive symptoms, and positive company. We did find significant mean differences for positive affect (t [197] = 2.56; p < .05), negative affect (t [197] = -2.22, p < .05).

Table 1 Descriptive Statistics and Correlations for Model Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>M</th>
<th>SD</th>
<th>N</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age</td>
<td>19.55</td>
<td>1.52</td>
<td>199</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Sex*</td>
<td>0.91</td>
<td>0.28</td>
<td>199</td>
<td>-0.07</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Baseline loneliness</td>
<td>1.80</td>
<td>0.48</td>
<td>199</td>
<td>-0.09</td>
<td>-0.02</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Depressive feelings</td>
<td>31.18</td>
<td>7.28</td>
<td>199</td>
<td>1.6*</td>
<td>-0.12</td>
<td>-0.56**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Positive affect</td>
<td>4.83</td>
<td>0.80</td>
<td>199</td>
<td>-0.10</td>
<td>-0.07</td>
<td>-0.43**</td>
<td>-0.44**</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Negative affect</td>
<td>1.55</td>
<td>0.58</td>
<td>199</td>
<td>0.08</td>
<td>0.07</td>
<td>0.50**</td>
<td>0.46**</td>
<td>-0.43**</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7. Positive company</td>
<td>6.07</td>
<td>0.60</td>
<td>199</td>
<td>-0.09</td>
<td>0.02</td>
<td>-0.38**</td>
<td>-0.27**</td>
<td>0.43**</td>
<td>-0.29**</td>
<td>-</td>
</tr>
<tr>
<td>8. Negative company</td>
<td>1.53</td>
<td>0.56</td>
<td>199</td>
<td>0.17*</td>
<td>-0.07</td>
<td>0.40**</td>
<td>0.27**</td>
<td>-0.32**</td>
<td>0.52**</td>
<td>-0.45**</td>
</tr>
</tbody>
</table>

Note: *0 = male; 1 = female. * p < .05. ** p < .01.
and negative company ($t[197]= -2.52; p < .05$), in that participants with a relationship compared to those without a relationship had higher mean levels of positive affect ($M[SD] = 4.98 [0.69]$ for participants with relationship, $M[SD] = 4.69 [0.85]$ for participants without relationship), and lower mean levels of negative affect ($M[SD] = 1.47 [0.48]$ for participants with relationship, $M[SD] = 1.65 [0.65]$ for participants without relationship), and negative company ($M[SD] = 1.43 [0.50]$ for participants with relationship, $M[SD] = 1.64 [0.61]$ for participants without relationship). In addition, we checked whether mean levels differed between participants who left home for college and participants who lived with their parents. Significant mean differences were found for depressive symptoms ($t[197]= 2.33; p < .05$) and negative company ($t[197]= 2.49; p < .05$) only, in that participants who left home for college had higher levels of depressive symptoms ($M[SD] = 32.09 [7.76]$) and negative company ($M[SD] = 1.60 [0.64]$) than participants living with their parents ($M[SD] = 29.62 [6.11]$ for depressive symptoms, $M[SD] = 1.42 [0.36]$ for negative company). Because of these differences, we controlled for relationship status and living situation in all models.

**Loneliness in Relation to Perceptions of Company and Affect**

First, an unconditional model was tested without predictors. Intraclass correlations were .49 for positive affect and .53 for negative affect, indicating that respectively 49% and 53% of the variance in positive and negative affect occurred at the individual level. Second, we examined relations between baseline loneliness and positive and negative perceptions of company. Loneliness was positively related to negative company ($B = 0.20$, $SE = 0.04$, $p < .001$) and negatively related to positive company ($B = 0.19$, $SE = 0.05$, $p < .001$). Hence, students with higher levels of loneliness perceived their company as more negative and less positive. Third, we tested whether baseline levels of loneliness were related to positive and negative affect (Table 2). Results showed that loneliness was significantly related to both positive and negative affect, in that students higher in loneliness had lower levels of positive affect and higher levels of negative affect.

**Hypersensitivity to Social Threat**

To test hypersensitivity to social threat, we first examined relations between negative company and affect, while controlling for affect at the previous assessment. Negative company was significantly related to higher levels of negative affect and lower levels of positive affect. Next, we tested whether loneliness moderated the relation between negative company and affect. We found that loneliness moderated the relation between negative company and positive affect, in that levels of positive affect in students with higher levels of loneliness were lower when they were in negative company, compared to students with low levels of loneliness (Figure 1). Additionally, loneliness moderated the relation between negative company and negative affect, in that students high in loneliness were more negatively affected by negative company than students low in loneliness (Figure 2).

### Table 2: Multivariate Multilevel Models for Relations Between Negative Company, Loneliness, and Negative and Positive Affect

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative affect</th>
<th>Positive affect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>$1.21^{**}$</td>
<td>$1.31^{**}$</td>
</tr>
<tr>
<td>Affect t-1</td>
<td>$1.18^{**}$</td>
<td>$1.31^{**}$</td>
</tr>
<tr>
<td>Sex</td>
<td>$1.18^{**}$</td>
<td>$1.31^{**}$</td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>$1.10^{**}$</td>
<td>$1.21^{**}$</td>
</tr>
<tr>
<td>Negative company</td>
<td>$1.10^{**}$</td>
<td>$1.21^{**}$</td>
</tr>
<tr>
<td>Loneliness</td>
<td>$1.10^{**}$</td>
<td>$1.21^{**}$</td>
</tr>
<tr>
<td>Loneliness x Company</td>
<td>$1.10^{**}$</td>
<td>$1.21^{**}$</td>
</tr>
<tr>
<td>Model summary</td>
<td>Deviance: $1.31^{**}$</td>
<td>$1.21^{**}$</td>
</tr>
<tr>
<td>Parameters</td>
<td>19</td>
<td>19</td>
</tr>
</tbody>
</table>

Note. All observation-level variables were group-mean centered, and all person-level variables were grand-mean centered. In all models, we controlled for relationship status, living situation, and age. $^{*} p < .05$, $^{**} p < .01$, $^{***} p < .001$.

**Figure 1** Relation between negative company and positive affect, split out for levels of loneliness.
For a measure to be included in the analyses, we chose a time frame of 20 minutes after the email was sent. As this excluded many measures, we checked whether the results would differ when we used a time frame of 30 minutes. The results were the same, except for the moderation of trait loneliness on the relation between negative company and negative affect, which became non-significant when we used all measures filled out within 30 minutes.

As we had only a small number of males in our sample, we checked whether the results would change if we excluded the males ($N = 19$) from the analyses. The only effect that changes is the moderation of trait loneliness on the relation between negative company and positive affect, the coefficient of this effect decreases slightly after exclusion of males, and becomes marginally significant ($B = -0.05$, $SE = 0.03$, $p = .08$).

4. For a measure to be included in the analyses, we chose a time frame of 20 minutes after the email was sent. As this excluded many measures, we checked whether the results would differ when we used a time frame of 30 minutes. The results were the same, except for the moderation of trait loneliness on the relation between negative company and negative affect, which became non-significant when we used all measures filled out within 30 minutes.

5. As we had only a small number of males in our sample, we checked whether the results would change if we excluded the males ($N = 19$) from the analyses. The only effect that changes is the moderation of trait loneliness on the relation between negative company and positive affect, the coefficient of this effect decreases slightly after exclusion of males, and becomes marginally significant ($B = -0.05$, $SE = 0.03$, $p = .08$).
Discussion

The main aim of the present study was to examine two characteristics of the socio-cognitive model on loneliness (Cacioppo & Hawkley, 2009), hypersensitivity to social threat and hyposensitivity to social reward, in the daily lives of late adolescents. We found support for hypersensitivity to social threat, in that students high in loneliness decreased more in positive affect when they perceived their company more negatively than students low in loneliness. For hyposensitivity to social reward, opposite effects were found, indicating that students high in loneliness benefited more from positive company than adolescents low in loneliness, as their levels of negative affect decreased more when they were in more positive company.

First of all, we found that students high in loneliness perceived their company as less positive and more negative, and experienced higher levels of negative affect and lower levels of positive affect than students low in loneliness. These effects remained significant while controlling for depressive symptoms and, importantly, depressive symptoms were not significantly related to positive and negative perceptions of company when loneliness was entered in the model, indicating that this effect is specific to feelings of loneliness. These findings are in line with previous ESM studies in early adolescents and students (Hawkley et al., 2007, van Roekel et al., 2013), that found that people high in loneliness have more negative and less positive perceptions of their company and of their interactions with others.

Regarding hypersensitivity to social threat, results are in line with our expectations that were based both on the socio-cognitive model (Cacioppo & Hawkley, 2009) and on the previous ESM study examining the same relations in early adolescents (van Roekel et al., 2013). Students high in loneliness were more negatively affected by negative company, in that their state levels of positive affect decreased more and their state levels of negative affect increased more when they were in negative company, compared to students low in loneliness.

The next aim of the study was to examine hyposensitivity to social reward. Our findings were in line with the ESM study in adolescents, in that students high in loneliness were more positively affected by positive company than adolescents low in loneliness (i.e., levels of negative affect decreased more in students high in loneliness when in more positive company). These findings are in contrast with the socio-cognitive model on loneliness which states that lonely individuals are less rewarded by social stimuli. These contrasting findings may be explained by differences between studies in how positive social stimuli and loneliness were measured. We measured subjective experiences of students by examining their perceptions of company, whereas the study that found support for hyposensitivity (Cacioppo et al., 2009) had more objective, but non-personalized measures of the social stimuli, that were the same for all participants (i.e., positive pictures of social situations). It could be that lonely individuals are not rewarded by stimuli that are generally perceived as positive, whereas they do experience reward when they themselves perceive their environment as positive, which was the case in our study. Importantly, in previous studies on depression similar patterns were found in laboratory studies versus ESM studies. In laboratory studies, it is typically found that depressed people are less responsive to positive stimuli (e.g., Sloan, Strauss, & Wisner, 2001), whereas in ESM studies, depressed people are found to be more rewarded by positive events than non-depressed people (Peeters, Nicolson, Berkhof, Delespaul, & deVries, 2003; Thompson et al., 2012). These findings highlight the importance of examining differences between these two methods of research. A possible way to examine this in further research is by combining the two methods in the same sample of participants. First, people can participate in an experiment in which responses of lonely people to objective measures of social stimuli are examined (e.g., vignettes of social inclusion, pictures of positive social stimuli). Next, participants can enroll in an ESM study similar to the present study, in which responses to subjective social situations are examined. In that way, it is possible to disentangle whether the difference in findings is due to the type of measure used.

In addition, it may be that this hyposensitivity comes into play when adolescents are chronically lonely or have severe levels of loneliness, which was the case in the study that found support for hyposensitivity (Cacioppo et al., 2009). Our findings are based on a continuous loneliness measure, which does not provide any information about the chronicity of loneliness. This could indicate that high loneliness levels that are not necessarily chronic, may serve as a motivational state that encourages people to restore their social relationships, which in turn leads to heightened sensitivity to both positive and negative social environments. This would be in line with the evolutionary theory on loneliness (Cacioppo, Hawkley, et al., 2006) that states that loneliness has an evolutionary function, as feelings of social pain motivates people to go out and restore their social relations, which increases their chances of survival. On the other hand, when feelings of loneliness are sustained and become chronic, this may result in hyposensitivity to social reward. Future research should disentangle if and when the hypersensitivity to social reward as our results showed turns into hyposensitivity. This can be done by conducting prospective studies, in which loneliness and sensitivity to social reward are measured over several years. In this way, it is possible to examine whether individuals who become lonely are hypersensitive to social reward and whether this changes into hyposensitivity when loneliness levels become chronic.

Strengths and Limitations

The main strength of the present study is that we used the Experience Sampling Method to examine the two characteristics of the socio-cognitive model, which has high ecological validity and lowers the recall bias. In addition, as we used the same measures as in a previous ESM study on adolescents, we were able to replicate those findings in a
sample of college students. Yet, there are some limitations that need to be addressed.

First, as most students enrolled in Psychology and Educational Science are female, only 9% of the sample in the present study was male. Therefore, it was not possible to examine sex differences. Future research is necessary to disentangle whether the sensitivity to both positive and negative environments is the same for males and females. Importantly, excluding the men from the sample did not affect the results generally, which indicates that our findings were not specific to either sex.

Second, our sample was a relatively normative sample, consisting of university students who are highly educated. Therefore, we cannot generalize our findings to late-adolescents attending other forms of education (i.e., secondary vocational education, higher professional education). Yet, we found similar results in an early adolescent sample, in which adolescents were included that attended lower preparatory secondary school for technical and vocational training or preparatory secondary school for professional education (van Roekel et al., 2013). Therefore, we might assume that results may be similar in different types of education in late-adolescents as well. Related to this, loneliness levels were relatively low in our sample (although comparable to those other students samples) and were only measured once. Therefore, we were not able to examine the effects of chronic levels of loneliness.

Third, we must acknowledge that our measure of hypersensitivity to social threat does not measure sensitivity to objective measures of social threat, but merely heightened responses to perceived social threat. Although this provides important information about how lonely adolescents perceive their environment and how they are affected by these perceptions, further research is necessary to examine how these responses to perceived social threat relate to sensitivity to objective measures of social threat.

Conclusions

In sum, the present study was able to replicate previous findings of a study in early adolescents (van Roekel et al., 2013) on the two characteristics of the socio-cognitive model in a sample of college students. Support was found for hypersensitivity to social threat, in that students high in loneliness were more negatively affected by negative perceptions of company. Results for hyposensitivity to social reward were in the opposite direction; adolescents high in loneliness were more positively affected by positive perceptions of company than adolescents low in loneliness. These findings could indicate that loneliness serves as a motivational state, that makes people more sensitive to their environment in order to restore their social relations.
Chapter 6
The negative company we keep: High levels of negative social experiences in early adolescents’ daily lives

Submitted as:
van Roekel, E., Ha, T., Verhagen, M., Kuntsche, E., Scholte, R. H. J., & Engels, R. C. M. E.
The negative company we keep: High levels of negative social experiences in early adolescents’ daily lives.
CHAPTER 6
HIGH LEVELS OF NEGATIVE SOCIAL EXPERIENCES IN EARLY ADOLESCENCE

Abstract
Adolescence is characterized by high emotional variability and increased social stress. However, few studies have examined relations between negative social experiences and mood in daily life. The aim of the present study was to examine when and with whom adolescents experienced social stress (i.e., peaks in negative company), and how these peaks affected their mood. Further, we examined whether loneliness moderated these relations. The Experience Sampling Method was used to measure positive and negative affect and peaks in negative company. Results showed most likely to experience peaks when they were with classmates (compared to family, friends, and others), during week days (compared to weekend days), and in the morning (compared to afternoon and evening). Contrary to our expectations, some adolescents never experienced peaks in negative company, and some adolescents were not affected by peaks in their mood levels. Lonely adolescents experienced higher peaks in negative company and responded more negatively to peaks, compared to non-lonely adolescents. Our findings provide new insights in the daily experience of social stress in adolescence.

Introduction
Adolescence is characterized by a turbulent emotional life (Larson & Ham, 1993), in that adolescents experience a broader range of emotions and more variable mood states than children or adults (Larson et al., 1980). Indeed, greater emotional fluctuations have been found to relate to emotional maladjustment in adolescents (Larson et al., 1990; Silk et al., 2011; Silk, Steinberg, & Morris, 2003). This increased emotional intensity and variability may be partly due to biological changes in adolescence as puberty starts, which has a great impact on mood via hormonal changes (Buchanan, Eccles, & Becker, 1992) and brain development (e.g., Forbes et al., 2011). In addition, early adolescence is also characterized by changes in interpersonal relationships. One of the most important developmental tasks during adolescence is to be able to develop satisfying relationships with peers and create more autonomy from parents (Buhrmester & Furman, 1987; Hartup, 1996). This substantial shift in social relationships during early adolescence has been proposed to underlie these intense emotional states. While previous studies have linked and theorized the importance of social relationships and emotions, little research has studied these links on a day-to-day level to get more insight in the proximal effects of negative social relationships on adolescents’ emotional life. Therefore this study will investigate with whom and when adolescents experience negative social stress and, more importantly, how social stress affects their mood in terms of negative and positive affect.

Additionally, the increased importance of changing relationships puts a strain on some adolescents who experience difficulties dealing with negative social relationships, which may result in increased feelings of loneliness in adolescence (Quälfé, Brown, et al., 2013). Although there is some indication that lonely adolescents are hypersensitive to social threat in that they are more reactive to negative social relationships by experiencing heightened negative affect (van Roekel et al., 2013), it is not clear whether they experience more frequent negative social relationships and consequently their mood is more negatively affected (i.e., differential exposure hypothesis) or whether they respond more negatively to these social stressors, but do not necessarily experience more social stress (i.e., differential reactivity hypothesis). Hence, it is important to examine whether loneliness affects the extent to which adolescents experience negative social stress, and whether lonely adolescents respond more strongly to negative social stress than non-lonely adolescents.

Negative Social Experiences in Adolescence
In general, adolescents are found to experience more stressful life events (e.g., Compas, Davis, & Forsythe, 1985; Ge, Lorenz, Conger, Elder, & Simons, 1994) and more daily negative events (Larson & Ham, 1993) than pre-adolescents. In addition to this heightened exposure to stressors, adolescents also respond more negatively to performance-related stress (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Stroud et al., 2009; Sumter, Bokhorst,
Miers, Van Pelt, & Westenberg, 2010) and rejection-related stress (Silk et al., 2012; Stroud et al., 2009). Further, Experience Sampling studies have shown that adolescents respond negatively to daily negative events in real life in their levels of positive and negative affect (e.g., Larson & Ham, 1993; Schneiders et al., 2006; van Roekel et al., 2013), and these negative responses are stronger in adolescents compared to pre-adolescents (Larson & Ham, 1993). However, several researchers have argued that these heightened responses in adolescents are normative (Dahl, 2004; Gunnar et al., 2009; Stroud et al., 2009), and may only become a risk when extreme levels of stress are experienced, or when adolescents experience an accumulation of stressors. Further, previous research in adults has shown that reactivity to daily stressors has a great impact on wellbeing ten years later (Charles, Piazza, Mogle, Sliwinski, & Almeida, 2013), which highlights the importance of examining responses to negative social experiences in daily life.

Therefore, the present study focused on examining extreme levels of social stress (i.e., peaks in negative company) and emotional responses to those peaks, in early adolescents. As only a few studies have examined characteristics of these daily negative events, a further aim of the present study was to examine when (i.e., type of day and time of day) and with whom (i.e., type of company) adolescents were most likely to experience extreme levels of social stress.

Loneliness

As the social lives of adolescents become increasingly complex, it is no surprise that feelings of loneliness are found to be particularly present in early adolescence (e.g., van Roekel, Scholte, et al., 2010). Loneliness is typically defined as the negative emotional response to an experienced discrepancy between the actual and desired quantity or quality of one’s social network (Perlman & Peplau, 1981). As loneliness is related to various negative physical and mental health consequences (Hawkley & Cacioppo, 2010), it is important to examine what the maintaining factors are.

Previous cross-sectional research has shown that loneliness is related to more negative perceptions of interaction quality (Duck et al., 1994), interaction partners (Jones et al., 1981), and of close others (Tsai & Reis, 2009). The main disadvantage of cross-sectional designs however, is that participants report perceptions of interactions and others retrospectively, which could bias their ratings. In addition, perceptions of others and interactions are likely to be context-dependent, which is difficult to distinguish in cross-sectional studies. Therefore, it may be more appropriate to use the Experience Sampling Method (ESM), which makes it possible to examine participants’ mood and perceptions while they are living their lives. Only two studies have examined relations between loneliness and perceptions of others or interactions in the daily lives of adolescents, and found that lonely adolescents perceive both their company as well as their interactions with others as more negative and less positive than adolescents low in loneliness (Hawkley et al., 2007; van Roekel et al., 2013).

Importantly, loneliness does not only affect how adolescents experience their social environment, it also moderates the relation between perceptions of the social environment and mood levels. Compared to adolescents low in loneliness, adolescents high in loneliness are found to be more negatively affected by negative perceptions of company, in that they experience higher levels of negative affect, and lower levels of positive affect when they are in negative company (van Roekel et al., 2013). These findings are in line with a socio-cognitive model on loneliness, that states that lonely people are characterized by hypersensitivity to social threat (Cacioppo & Hawkley, 2009). However, these previous findings only indicate that lonely adolescents respond more negatively to negative social experiences, but not whether they are also exposed to more negative social experiences than non-lonely adolescents. Further, although these studies shed light on how lonely adolescents respond to higher or lower levels of negative company, very little is known about extreme levels of negative company. Hence, a further aim of the present study was to examine whether loneliness was related to heightened exposure to negative social experiences (i.e., more peaks in negative company; differential exposure hypothesis) and whether loneliness affected how adolescents responded to a peak (i.e., differential reactivity hypothesis).

The Present Study

In sum, the main aim of the present study was to examine within-person extreme levels of negative company (i.e., peaks in negative company) in early adolescents, by using the Experience Sampling Method. We first examined how often adolescents experienced peaks in negative company, and whether this could be predicted by individual characteristics, such as loneliness. Further, we aimed to investigate whether assessment-level characteristics (e.g., type of day, time of day, type of company) were related to the experience of a peak in negative company. Finally, we examined whether adolescents’ levels of positive and negative affect were affected by a peak in negative company and whether the extent to which adolescents were affected by a peak was moderated by loneliness. We expected that lonely adolescents would experience more peaks than non-lonely adolescents. As previous research has shown that state levels of loneliness are highest during week days and during assessments with classmates (van Roekel et al., 2013), we further hypothesized that adolescents would experience most peaks during week days and when they were with classmates.

Method

Participants

Adolescents were recruited through high schools in the Eastern part of the Netherlands. When the school agreed to participate (N = 4), all second year students (N = 933) were sent an information letter in which they were asked to participate. When they agreed to
participate, they had to return a consent form, signed by both themselves and their parents. A total group of 339 adolescents (36.33%) returned the consent forms. Due to organizational issues, illness, or withdrawal of consent, a few adolescents (N = 36) could not participate. Our final sample consisted of 303 adolescents (59.1% girls) with a mean age of 14.20 (SD = 0.54). Educational levels were all well represented in the sample: 23.4% of the adolescents attended preparatory secondary school for technical and vocational training, 35.8% attended preparatory secondary school for college, and 40.8% attended preparatory secondary school for university. The majority of the adolescents (97.1%) were born in the Netherlands.

Our sample consisted of 303 adolescents with 10,865 momentary assessments. We excluded adolescents from the analyses that filled out less than one-third of the total number of assessments (i.e., less than 18 assessments, N = 17, 561%). In addition, as we only measured negative company when adolescents were with others, we excluded adolescents that had too few assessments in company (i.e., less than 10 assessments in company, N = 8, 264%). This resulted in a final sample of 278 adolescents. The present study was approved by the Medical Ethical Committee (CMO Amhem-Nijmegen, 2009, No. 285). We checked whether participants that were retained differed from dropouts on model variables. Participants had slightly lower average levels of negative company (t [300] = 4.056, p < .001) and negative affect (t [301] = 4.056, p < .001) than dropouts. No differences were found between participants and dropouts for positive affect, loneliness, and sex (p > .05).

Procedure

The study consisted of a baseline questionnaire and the Experience Sampling Method (ESM) period. For a detailed description of the procedure, see (van Roekel et al., 2013). The baseline questionnaire was administered online during school hours. The ESM period took place three to eight weeks after the baseline questionnaire and always started on Fridays. Adolescents received a smartphone, on which a program was installed (http://myexperience.sourceforge.net) that emitted buzzing signals at nine random time points each day, for six consecutive days. When adolescents received a signal, they had to immediately pause their activity and fill out the questionnaire on the smartphone. When adolescents did not respond within two minutes after the signal, the buzzing signal was emitted again, with a maximum of three reminders. Data were stored on the smartphones and a text message was sent to the principal investigator after each completed questionnaire, making it possible to check compliance. Adolescents received the full reward of € 20 (i.e., about 27 US $) when they completed at least 55% of the momentary assessments.

Baseline assessments.

Trait loneliness. Trait levels of loneliness were measured with the peer-related subscale of the Louvain Loneliness Scale for Children and Adolescents (LLCA, Marcoen et al., 1987). This scale consisted of 12 items, which had to be rated on a 4-point scale, ranging from (1) never to (4) often. A sample item was “I think I have fewer friends than others have”. Cronbach’s alpha was .88.

Experience Sampling assessments.

Peaks in negative company. When adolescents were not alone, they were asked to rate the extent to which they perceived their company as threatening and judging (i.e., I feel threatened by this company and I feel judged by this company) on a scale ranging from (1) not at all to (7) very much. Responses on these two items were averaged to represent negative company.

An individual had a peak in negative company when he or she experienced an increase in negative company of at least one standard deviation above their own mean level of negative company across all assessments. Peaks were calculated based on each individuals’ mean level of negative company. First, we determined the cutoff point for the peaks in negative company, by calculating the aggregated mean and standard deviation over all momentary assessments, for each individual separately. Based on this cutoff point, we created a new dummy variable that represented for each assessment whether adolescents had a peak in negative company (score 1) or not (score 0). This is illustrated in Figure 1, in which all assessments are depicted for one participant. The dotted line indicates the cutoff-point (i.e., one standard deviation above the mean of negative company for this person).

Materials

Baseline assessments.

Trait levels of loneliness were measured with the peer-related subscale of the Louvain Loneliness Scale for Children and Adolescents (LLCA, Marcoen et al., 1987). This scale consisted of 12 items, which had to be rated on a 4-point scale, ranging from (1) never to (4) often. A sample item was “I think I have fewer friends than others have”. Cronbach’s alpha was .88.

Experience Sampling assessments.

Peaks in negative company. When adolescents were not alone, they were asked to rate the extent to which they perceived their company as threatening and judging (i.e., I feel threatened by this company and I feel judged by this company) on a scale ranging from (1) not at all to (7) very much. Responses on these two items were averaged to represent negative company.

An individual had a peak in negative company when he or she experienced an increase in negative company of at least one standard deviation above their own mean level of negative company across all assessments. Peaks were calculated based on each individuals’ mean level of negative company. First, we determined the cutoff point for the peaks in negative company, by calculating the aggregated mean and standard deviation over all momentary assessments, for each individual separately. Based on this cutoff point, we created a new dummy variable that represented for each assessment whether adolescents had a peak in negative company (score 1) or not (score 0). This is illustrated in Figure 1, in which all assessments are depicted for one participant. The dotted line indicates the cutoff-point (i.e., one standard deviation above the mean of negative company for this person).
**Positive and negative affect.** Positive and negative affect were measured by five items each. These items were selected from items used in other ESM studies (e.g., Peeters, Berkhof, Delespaul, & Rottenberg, 2006; Wichers et al., 2007). Positive affect was measured by the items joyful, satisfied, happy, energetic, and cheerful. Negative affect was measured by the items insecure, anxious, worried, low, and guilty. Adolescents had to rate to what extent they experienced the described emotion on a 7-point scale ranging from (1) not at all to (7) very much. Cronbach’s alphas for both positive and negative affect were calculated for each momentary assessment separately, and then averaged over all momentary assessments, which resulted in an alpha of .84 for positive affect and .71 for negative affect.

**Type of day.** To examine whether peaks were more likely to occur on week or weekend days, we created a dummy variable that represented the type of day (i.e., 0 = weekend day, 1 = week day).

**Time of day.** For the time of day, we created different dummy variables that represented whether an assessment occurred during the morning (i.e., morning = 1, afternoon and evening = 0), afternoon (i.e., afternoon = 1, morning and evening = 0), or evening (evening = 1, morning and afternoon = 0).

**Type of company.** When adolescents were with others, we asked them to describe who their company was (i.e., Who are you with?). These responses were coded to represent family (e.g., parents, siblings), friends, classmates, or others (e.g., team mates, teachers). To calculate the interrater reliability, 10% of the total number of assessments in company (N = 676) were randomly selected and coded by a different rater. This resulted in a kappa of .97 (p < .001), indicating good interrater reliability.

**Strategy of Analyses**

First, we calculated peaks in negative company, as was described in the Materials section. As some participants did not have a peak in negative company, we conducted independent sample t-tests to examine whether participants with peaks differed from participants without peaks on demographic variables and loneliness. The group without peaks in negative company was excluded from subsequent analyses. For the descriptive analyses, we then computed the total number of experienced peaks over the six day sampling period, and subsequently we examined correlations at the individual level between the number of peaks, the average level of their peaks, sex, and loneliness. Second, we examined when and in which company adolescents experienced peaks. As the momentary assessments (Level 1) were nested within individuals (Level 2), we conducted multilevel logistic regression analyses. In these analyses, the outcome variable was whether or not adolescents experienced a peak in negative company at a given assessment during the total sampling period. Type of day (week, weekend), time of day (morning, afternoon, evening), and type of company (family, friends, classmates, others) were recoded into dummy variables and entered in the model as predictors for the variable representing the peaks.

Third, we examined how adolescents’ mood, that is negative and positive affect, was affected by negative social experiences. In other words, we examined whether positive and negative affect differed between assessments with a peak and assessments without a peak. Because positive and negative affect were correlated (r = -.46), we used multivariate multilevel modeling, in which positive and negative affect were simultaneously included as dependent variables. Further, we included the cross-level interaction with trait loneliness, to examine whether high lonely adolescents had different levels of affect in response to a peak, compared to low lonely adolescents, while controlling for sex.

**Results**

First, we calculated the cutoff point for a peak in negative company for each participant and determined which assessments reflected a ‘peak moment’ in negative company. Some of the participants never experienced a peak in negative company during the assessment period (i.e., their levels of negative company did not exceed their cutoff point, N = 3, 1.08%), and some participants never rated their company as negative (i.e., always filled out the lowest score on the negative company items, N =22, 7.91%). As these adolescents could not be included in further analyses, we examined whether the adolescents that experienced peaks differed from adolescents that did not experience peaks on sex and loneliness. We found differences between the groups in sex, in that boys were overrepresented in the group that did not experience peaks (χ² = 3.19, p <.03). No differences were found between the groups in levels of loneliness.

In the group that experienced peaks (N = 253), we further examined the descriptive statistics of the model variables (Table 1). Mean levels of loneliness were relatively low, given the theoretical range (i.e., 12-48). The number of peaks adolescents had over all sampling days ranged between 1 and 13, with an average of 3 peaks. Next, we calculated correlations between all variables. As can be seen in Table 1, sex correlated positively with loneliness and the number of peaks, indicating that girls had higher levels of loneliness and experienced more peaks in negative company. In addition, loneliness was positively related to the average level of the peaks, in that adolescents high in loneliness on average experienced higher level of peaks in negative company than adolescents low in loneliness. Loneliness was positively related to average levels of negative affect and negatively related to average levels of positive affect.

**Characteristics of Peaks in Negative Company**

Next, we examined when and with whom adolescents experienced peaks, by using a multilevel logistic regression model (see Table 2). First, we examined whether type of day (i.e., week versus weekend) was related to whether or not adolescents experienced a peak on a given moment during the assessment period. We found a significant relation...
between type of day and having a peak, which indicated that participants were almost three times more likely to experience a peak on weekdays than on weekend days.

Subsequently, we entered time of day as a predictor in the model. First, we used assessments in the morning as a reference category, and included dummy variables representing assessments in the afternoon and evening. Significant relations were found between the dummy for afternoon and having a peak and the dummy for evening and having a peak. This indicated that when an assessment took place in the afternoon or evening compared to morning, the odds of experiencing a peak decreased by a factor of 0.52 or 0.25, respectively. Next, we entered dummy variables for morning and evening in the model, so that afternoon assessments were the reference category. We found a significant relation between the dummy variable for evening and having a peak. This finding implied that when assessments took place in the evening, compared to the afternoon, the odds of experiencing a peak decreased by a factor of 0.47.

Finally, we examined the relation between type of company and experiencing a peak. First, we used the company of family as the reference category, by adding dummy variables representing the company of friends, classmates, and others. All dummy variables were significant predictors for having a peak. For situations with friends, the odds of having a peak were 1.82 higher than for situations with family. Regarding situations with classmates, the odds of experiencing a peak increased with 4.50, compared to situations with family. For situations in which adolescents were with others, the odds of experiencing a peak increased with 2.81, compared to situations with family. In order to examine differences between friends and the other categories, we entered dummy variables for family, classmates, and others to the model. Significant relations were found only between the dummy variable for classmates and having a peak, indicating that in situations when adolescents were with classmates, the odds of experiencing a peak were 2.47 times higher than in situations in which adolescents were with friends. No relation was found between the dummy variable for others and having a peak, indicating that the odds of experiencing a peak was similar for situations with friends and others. Lastly, to examine differences between situations with classmates and others, we included dummy variables representing situations with family, friends, and others to the model. The dummy variable representing situations with others was related to having a peak, which indicated that in situations with others, the odds of experiencing a peak were 0.62 times lower than in situations with classmates.

Table 1 Descriptive Statistics and Correlations for Model Variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M (SD)</th>
<th>Range</th>
<th>N</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sex*</td>
<td>0.62 (0.49)</td>
<td>0 - 1</td>
<td>252</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2. Loneliness</td>
<td>17.80 (5.38)</td>
<td>12 - 46</td>
<td>247</td>
<td>.18**</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Number of peaks</td>
<td>3.61 (2.45)</td>
<td>1 - 17</td>
<td>252</td>
<td>.18**</td>
<td>.10 -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Average value of peak</td>
<td>2.99 (1.09)</td>
<td>1.50 - 7.00</td>
<td>252</td>
<td>.04</td>
<td>.27***</td>
<td>.02</td>
<td>.39***</td>
<td></td>
</tr>
<tr>
<td>5. Negative affect</td>
<td>1.47 (.44)</td>
<td>1.00 - 3.83</td>
<td>251</td>
<td>.16*</td>
<td>.27***</td>
<td>.02</td>
<td>.39***</td>
<td></td>
</tr>
<tr>
<td>6. Positive affect</td>
<td>5.16 (.70)</td>
<td>3.22 - 6.58</td>
<td>251</td>
<td>.04</td>
<td>.31***</td>
<td>.06</td>
<td>.25***</td>
<td>.46***</td>
</tr>
</tbody>
</table>

Note. *0 = boy; 1 = girl. ** *p < .05. *** p < .01. **** p < .001.

Table 2 Odds Ratios (95% confidence interval in brackets) of the Multilevel Logistic Regression Model Predicting Peaks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of day</td>
<td></td>
</tr>
<tr>
<td>Weekend versus week</td>
<td>2.97***</td>
</tr>
<tr>
<td>(2.36 – 3.76)</td>
<td></td>
</tr>
<tr>
<td>Time of day</td>
<td></td>
</tr>
<tr>
<td>Morning versus afternoon</td>
<td>0.52***</td>
</tr>
<tr>
<td>(0.44 – 0.60)</td>
<td></td>
</tr>
<tr>
<td>Morning versus evening</td>
<td>0.25***</td>
</tr>
<tr>
<td>(0.19 – 0.33)</td>
<td></td>
</tr>
<tr>
<td>Afternoon versus evening</td>
<td>0.48***</td>
</tr>
<tr>
<td>(0.37 – 0.63)</td>
<td></td>
</tr>
<tr>
<td>Type of company</td>
<td></td>
</tr>
<tr>
<td>Family versus friends</td>
<td>1.82***</td>
</tr>
<tr>
<td>(1.33 – 2.51)</td>
<td></td>
</tr>
<tr>
<td>Family versus classmates</td>
<td>4.50**</td>
</tr>
<tr>
<td>(3.31 – 6.13)</td>
<td></td>
</tr>
<tr>
<td>Family versus others</td>
<td>2.81***</td>
</tr>
<tr>
<td>(1.75 – 4.51)</td>
<td></td>
</tr>
<tr>
<td>Friends versus classmates</td>
<td>2.47**</td>
</tr>
<tr>
<td>(1.88 – 3.24)</td>
<td></td>
</tr>
<tr>
<td>Friends versus others</td>
<td>1.54</td>
</tr>
<tr>
<td>(0.98 – 2.42)</td>
<td></td>
</tr>
<tr>
<td>Classmates versus others</td>
<td>0.62*</td>
</tr>
<tr>
<td>(0.41 – 0.95)</td>
<td></td>
</tr>
</tbody>
</table>

Note. * p < .05. ** p < .01. *** p < .001.

Negative and Positive Affect at Peak Moments

Finally, we examined whether positive and negative affect differed between peak moments versus non-peak moments. First, we calculated mean levels of positive and negative affect during peak moments and non-peak moments (Table 3). Paired samples t-tests showed that negative affect was significantly higher during peak moments (t[250] = -6.62, p < .001) and positive affect was significantly lower during peak moments, compared to non-peak moments (t[250] = 3.69, p < .001). Next, we examined the relations between a dummy variable representing whether participants experienced a peak (1) or not (0) at a given assessment, and their levels of positive and negative affect at that assessment, by using multivariate multilevel modeling. Negative affect was significantly higher on peak moments (β = 2.0, SE = .03, p < .001), compared to non-peak moments, whereas positive affect was significantly lower on peak moments (β = -1.5, SE = .04, p < .001).
not in line with our expectations, we further examined whether the adolescents that were not affected by a peak (i.e., had a score of 1 on NA at peak moments; \( N = 88 \)) differed from the adolescents that were affected by a peak (i.e., had a score of > 1 on NA at peak moments; \( N = 164 \)). We found a significant difference in levels of loneliness between the two groups (\( t(245) = -2.35, p < .05 \)), in that adolescents who were not affected (\( M[SD] = 16.71[5.25] \)) had lower levels of loneliness than adolescents who were affected by a peak (\( M[SD] = 18.38[5.38] \)). In addition, we found differences between the groups in sex, in that boys were overrepresented in the group that was not affected by a peak (\( \chi^2 = 4.61, p < .05 \)).

**Discussion**

The main aims of the present study were to examine (a) when and with whom adolescents experienced peaks in negative company, (b) how adolescents responded to those peaks in their levels of positive and negative affect, and (c) whether loneliness was related to the experience of peaks and to the affective responses to these peaks. Our results showed that adolescents were more likely to experience peaks on weekdays, during mornings, and with classmates. Adolescents showed negative affective responses to peaks, which was amplified in lonely adolescents. Surprisingly, some adolescents did not experience peaks in negative company during the sampling period, and some adolescents were not negatively affected by peaks in negative company.

**Characteristics of Peaks**

As no studies have previously examined extreme negative social situations in adolescents’ daily lives, we first examined whether adolescents experienced peaks during the sampling period. Except for a small group that never experienced peaks (\( N = 25 \)), most adolescents did experience an extreme negative social situation (with an average of 3 peaks), which may indicate that experiencing peaks in negative company is normative in early adolescence (Larson & Ham, 1993). As expected, these peaks were more likely to occur on weekdays, compared to weekend days, and were most likely to occur with classmates, compared to the other types of company. These findings are not surprising, as previous research has shown that adolescents experience the highest levels of state loneliness and negative affect when they are with classmates (van Roekel et al., 2013; van Roekel, Verhagen, Engels, Goossens, & Scholte, submitted), which is most often on weekdays. Further, we found that peaks were most likely to occur during mornings, compared to afternoons and evenings. This may be in line with the other findings, in that adolescents are likely to enter the company of classmates in the morning, which may lead to an increase in negative company.

Interestingly, we found that adolescents were least likely to experience peaks in situations with family, compared to the other types of company. More specifically, our

**Table 3** Descriptive Statistics of Negative and Positive Affect

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( M[SD] )</th>
<th>Range</th>
<th>( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative affect Peak</td>
<td>1.73 (0.81)</td>
<td>1.00 – 7.00</td>
<td>251</td>
</tr>
<tr>
<td>Negative affect Non-peak</td>
<td>1.45 (0.44)</td>
<td>1.00 – 3.95</td>
<td>251</td>
</tr>
<tr>
<td>Positive affect Peak</td>
<td>4.99 (0.98)</td>
<td>1.00 – 7.00</td>
<td>251</td>
</tr>
<tr>
<td>Positive affect Non-peak</td>
<td>5.17 (0.71)</td>
<td>3.05 – 5.17</td>
<td>251</td>
</tr>
</tbody>
</table>

**Figure 2** Levels of negative affect during peak moments and non-peak moments, split for loneliness.
findings showed that adolescents were almost two times more likely to experience peaks in negative company with friends, compared to family. Hence, although previous research has shown that time spent with friends is related to higher levels of positive affect compared to time spent with family (Larson, 1983; van Roekel et al., 2013), our findings show that this time with friends may also be experienced negatively, as adolescents are more likely to experience higher levels of judging and threatening company in situations with friends. A possible explanation for this finding is that family may represent a relatively safe haven for adolescents, compared to friends. Although the time spent with family may not always be experienced as positive and exciting, family is not very likely to be judging and threatening to adolescents. Even though friendships are voluntary relationships and adolescents can choose who they spend their time with, previous studies have shown that friendships also contain high levels of conflict (Laursen, 1993). Friendships are different from parental relationships, as they may require more skills to for example maintain or increase popularity, or have high quality relationships (affiliative and supportive functions of friendships). As a result, friendships may trigger more rewarding experiences on the one hand, but when these needs are not met may cause more stress and thus negative emotions.

**Affective Responses to Peaks**

As expected, our findings regarding the affective responses to peaks in negative company show that adolescents experience higher levels of negative affect and lower levels of positive affect during peak moments. However, our additional analyses indicated that there was a substantial group of adolescents (i.e., 35% of the total sample) that was not affected by a peak in negative company, in that they had the lowest possible levels of negative affect at peak moments. This indicates that some adolescents did not experience negative affect, even though they did experience a peak in negative company. This was surprising, as we expected based on previous research (e.g., Gunnar et al., 2009; Stroud et al., 2009) that it would be normative for adolescents to respond negatively to negative social experiences. Importantly, the previous studies examining affective responses to negative daily experiences only examined direct relations between these two variables, and did not further specify whether there was a subgroup of adolescents that did not respond to negative events (Larson & Ham, 1993; Schneiders et al., 2006). Hence we do not know whether there may have been a subgroup of non-responders in those studies as well. A possible explanation for this finding may be that although adolescents are found to experience within-person extreme levels of negative company, these levels may still be relatively low and for some adolescents, may not be perceived as very negative situations. Hence, it could be that the experience of being judged for example, is not an unusual situation for adolescents, and therefore they may not feel worse when they experience extreme levels of judging company for example. In this way, experiencing peaks may be normative in adolescence, which may be why a relatively large group was not affected in their mood by those peaks. Further research is necessary to examine why this subgroup does not respond to peaks, and whether this group would respond to more extreme negative social situations.

**Loneliness**

One of the further aims of the present study was to examine whether loneliness was related to the experience of peaks, and whether loneliness moderated the relation between the experience of peaks and positive and negative affect. Our findings showed that lonely adolescents on average had higher peaks than low lonely adolescents. This is in line with a previous study that found that higher levels of loneliness were related to higher levels of negative company (van Roekel et al., 2013). Importantly, loneliness was not related to the number of peaks adolescents experienced. In other words, although lonely adolescents did experience higher peaks, they did not experience more peaks than non-lonely adolescents, indicating that they are more affected by negative social experiences but are not exposed to more negative social experiences. These findings show that the differential exposure hypothesis does not hold, which is in line with previous research (Cacioppo et al., 2003). In addition, we found that loneliness was related to the affective responses to peaks, in that lonely adolescents experienced higher levels of negative affect in response to a peak than non-lonely adolescents. These findings provide evidence for the differential reactivity hypothesis (Cacioppo et al., 2003), which states that lonely individuals experience more stress in general because they respond more negatively to stressors than non-lonely people.

**Sex Differences**

When we further examined whether the group that did not experience peaks differed from the group that did experience peaks, we found that boys were more likely than girls to be part of the group without peaks. Further, boys were also more likely to be non-responders compared to girls. These findings indicate that boys may experience lower levels of negative social experiences than girls, and that boys also respond less to peaks in negative company. These sex differences may not be very surprising, as previous research has often showed that boys and girls experience social relationships differently (for review, see Rose & Rudolph, 2006), in that girls value intimacy and closeness in relationships more than boys, and girls spend more time in social contexts than boys (Larson & Richards, 1991).

**Strengths and Limitations**

One of the strengths of the present study is that we used the ESM, which made it possible to examine negative social experiences in real life. Further, as we had multiple assessments within individuals, we were able to examine within-individual extreme negative social experiences. However, some limitations need to be acknowledged. First of
all, as our findings showed that around one-third of the adolescents did not report changes in mood in response to peaks, this indicated that average scores on negative company may have been relatively low, and not all adolescents experienced high levels of threatening and judging company. However, because we calculated within-individual extremes, a peak did represent an extreme level of negative company for that specific individual. Further research may investigate peaks by using different items, that measure for example the extent to which adolescents feel (un)close to their company, or dislike their company, which may be more common experiences in daily life.

Second, as we used a normative sample, levels of loneliness were relatively low, and most adolescents did not experience extremely high levels of negative company. It would be interesting to examine in further research whether adolescents with chronic levels of loneliness for example, experience a greater number of peaks, and respond differently to those peaks from adolescents with transient levels of loneliness. Further, we do not know whether the heightened responses of lonely adolescents found in the present study further increased their loneliness levels. Further research is necessary to investigate how the affective responses to peaks may influence future levels of loneliness.

Conclusions

Our findings showed that adolescents were more likely to experience peaks in negative company when they were with classmates (compared to family, friends, and others), during weekdays (compared to weekend days), and in the morning (compared to afternoon and evening). Our findings further showed that adolescents high in loneliness experience higher levels of negative company and respond more negatively to peaks in negative company than low lonely adolescents. Unexpectedly, we found a relatively large group of adolescents that did not show affective responses to peaks in negative company, as they experienced the lowest possible levels of negative affect during all peak moments. These findings may indicate that the experience of peaks in negative company is normative and adolescents and therefore adolescents do not respond to peaks in their levels of negative affect.
Part II
Genetic Influences
Chapter 7
Loneliness in adolescence: Gene - environment interactions involving the serotonin transporter gene

Published as:
CHAPTER 7

5-HTTLPR GENE IN RELATION TO TRAIT LONELINESS

Abstract

Loneliness is assumed to peak in early adolescence and to decrease throughout middle and late adolescence, but longitudinal confirmation of this tendency is lacking. Behavioral genetic studies with twin designs have found a significant genetic component for loneliness in children and adults, but no molecular genetic studies have been conducted to reveal the functional polymorphisms involved. Associations among the serotonin transporter gene (5-HTTLPR), sex, parental support, and loneliness were examined in a longitudinal study spanning five annual waves (N = 306). Using latent growth curve modeling (LGCM), loneliness was found to be highest in early adolescence and slowly declined throughout adolescence. The 5-HTTLPR genotype was related to the development of loneliness, in that short allele carriers remained stable in loneliness over time, whereas adolescents with the long-long genotype decreased in loneliness. Interactions were found between maternal support and 5-HTTLPR genotype, showing that adolescents who perceived little support from their mothers and carried a short allele were at increased risk for developing loneliness. Our study is the first to chart adolescent loneliness longitudinally and to examine the genetic underpinnings of loneliness. Our results contribute to a further understanding of the environmental and genetic basis of loneliness. Replication of our results is needed in both population-based and clinical samples.

Introduction

Loneliness is defined as the negative emotional response to the discrepancy between the actual and desired quantity and quality of one’s social network (Perlman & Peplau, 1981). Because loneliness is related to various mental and physical health problems such as anxiety, schizophrenia, depression, sleep disturbance, poorer immune functioning, and cardiovascular disease (Heinrich & Gullone, 2006), it is important to examine both its developmental course and its antecedents. The present study is the first to chart changes in loneliness in adolescence and examined the roles of both genes and perceived parental support as predictors of loneliness during this phase of life.

The Developmental Course of Loneliness in Adolescence

Loneliness can be experienced from early childhood on, but is found to peak during early adolescence (Heinrich & Gullone, 2006). A possible explanation for this increase in loneliness can be that early adolescence is a turbulent period in which peers become increasingly important, the self is mainly defined in terms of one’s social relationships (Parkhurst & Hopmeyer, 1999), and young people make the transition from primary school to secondary school, which leads to temporary disruption of the social network. Because attachment and identity issues are gradually resolved during adolescence and new friendships are formed in secondary school, one may expect that loneliness declines slowly throughout middle and late adolescence. Cross-sectional studies using age cohorts indeed suggest a decline in loneliness from early to late adolescence (e.g., Marcoen & Goossens, 1993). Although such cross-sectional studies provide valuable information about the developmental trends in levels of loneliness, longitudinal data are necessary to examine intra-individual changes in loneliness.

Genes and Loneliness

Behavioral genetic studies using twin designs have found a significant genetic component for loneliness in children and adults, with estimates ranging between 48% and 55% (Boomsma et al., 2005; McGuire & Clifford, 2000). No molecular-genetic studies have been conducted to find polymorphisms involved in loneliness. In the present study, we examined the relations between loneliness in adolescence and 5-HTTLPR, a functional polymorphism in the promoter region of the serotonin transporter gene (5-HTT). The 5-HTTLPR genotype is a variable repeat sequence in the promoter region of the gene, which encodes two allelic variants: a short allele and a long allele. Carrying the short allele of the 5-HTTLPR genotype may be a susceptibility factor, which can lead to mental problems if and when negative environmental conditions apply (the ‘double hit’ hypothesis, Murphy et al., 2008). Up to now, no studies have examined the relation between loneliness and the 5-HTTLPR genotype. In previous research, however, this polymorphism has been linked to depressive symptoms, which are highly correlated with...
loneliness (Weeks, Michela, Peplau, & Bragg, 1980), and to shyness, which is a precursor for loneliness (N. A. Fox et al., 2005).

5-HTTLPR Genotype in Depression

Direct effects of the 5-HTTLPR genotype have been found rather rarely in adult samples, with short alleles being more prevalent in depressive patients (Hauser et al., 2003; Hoefgen et al., 2005), and with the short-short genotype being significantly related to depressive outcomes (Cervilla et al., 2006; Kaufman et al., 2004). However, significant gene-environment interactions, in which the 5-HTTLPR genotype interacts with negative environmental factors, such as life stress, and predicted depression in adults, have been obtained somewhat more consistently (for review, see Munafò et al., 2009).

The study of Eley et al. (2004) found a significant direct effect of the 5-HTTLPR genotype on depression in adolescence for girls only, whereas the Sjöberg et al. (2006) study found no such effects for either gender. Both studies found significant gene-environment interactions with adverse life events and conflicts in the family and psychosocial risks, respectively, as negative environmental conditions, in girls only. In both cases, female short allele carriers were at greater risk for depression in stressful environments.

Presumed Biological Mechanisms

In recent years, an increasing number of studies have tried to disentangle the biological mechanisms underlying the relation between the 5-HTTLPR genotype and depression. These studies showed that carrying the short allele was a risk factor for problems with negative affect regulation. The first line of studies examining the biological mechanism investigated the role of the 5-HTTLPR genotype in neural activation in response to emotional stimuli (e.g., fearful and angry faces). Results indicated that short allele carriers displayed stronger amygdala activation in response to fearful stimuli, compared to long allele carriers (Heinz et al., 2005; Pezawas et al., 2005). This overactivation of the amygdala may reflect oversensitivity to threat-related signals.

The second line of research in this area revealed reduced connectivity (or reduced functional coupling) in carriers of the short allele between the amygdala and the perigenual anterior cingulate cortex (pACC) (Pezawas et al., 2005). Because the coupling between these two brain structures is conceptualized as the feedback circuit involved in the extinction of negative affect, a loss of functional integration between these areas can lead to less inhibitory regulation of the amygdala. In addition, short allele carriers also showed greater connectivity between the amygdala and the ventromedial pre-frontal cortex (vmPFC) (Heinz et al., 2005). The latter type of increased functional coupling does not necessarily indicate a greater risk for psychopathology, but rather suggests a compensatory effort of the vmPFC to regulate the overactivated amygdala. The same underlying biological mechanism may play a role in the development of loneliness as well. Because loneliness is defined as the negative emotional response to the discrepancy between one’s actual and desired social relationships, one may expect that the 5-HTTLPR genotype is also relevant in loneliness.

Environmental Factors

Environmental effects, such as negative life events (Paykel, 2003), negative parenting, and maltreatment (Alloy, Abramson, Smith, Gibb, & Neeren, 2006), have a direct influence on depression and related problems. High perceived parental support, one particular aspect of effective parenting, is related to lower levels of loneliness (Franzoi & Davis, 1985). Perceived support from primary caregivers (mostly mothers) has also been found to interact with the 5-HTTLPR genotype in predicting behavioral inhibition and depression in children (Fox et al., 2005; Kaufman et al., 2004). In short, one may expect that perceived parental support is related to loneliness, and that it interacts with the 5-HTTLPR genotype in predicting loneliness.

The Present Study

The aim of the present study was to test the relation between the 5-HTTLPR genotype and the onset and development of loneliness, using a longitudinal five-wave design. Subsequently, we examined whether perceived parental support is related to the onset and development of loneliness and whether the 5-HTTLPR genotype interacts with parental support in predicting loneliness. We hypothesized that loneliness will be highest in early adolescence, and will slowly decrease throughout adolescence. We expected the short allele of the 5-HTTLPR genotype and low levels of maternal and paternal support to be related to higher onset and slower decrease of loneliness. Furthermore, we expected the 5-HTTLPR genotype to interact with parental support in predicting loneliness, such that short allele carriers who receive low levels of parental support demonstrate the highest levels of loneliness.

Method

Participants and Procedure

Data for the present study were derived from the longitudinal Dutch survey study ‘Family and Health’, which examines different family processes in relation to various health behaviors in adolescence (Harakeh, Scholte, de Vries, & Engels, 2005). Addresses of families with at least two children, aged 13–16 years, were derived from registers of 22 municipalities. The families were sent a letter in which they were invited to participate. Of the responding 885 families who fulfilled the criteria, parents were married or living together, all family members were biologically related to each other, and participating siblings were neither twins nor mentally of physically disabled; 428 families were selected to obtain an equal distribution of sibling dyads (boy–boy, girl–girl, boy–girl), and an equal division of educational levels.
Only the data of the youngest adolescent in each family were used, because these adolescents were entering adolescence at T1, thus allowing us to examine changes in loneliness at the start of adolescence. At the first wave, the mean age of these adolescents was 13.4 (SD = 5.0), 53.3% were girls. The age range at the successive waves was 12–14 years (T1), 13–15 years (T2), 14–16 years (T3), 15–17 years (T4), and 16–18 years (T5). At any given point in time throughout the study, the age range (youngest – oldest participant) was 1 year and 9 months. One-third of the adolescents attended lower education, one-third intermediate general education, and one-third attended the highest level of secondary school. The number of drop-outs in subsequent waves was low, with the number of participating families ranging between 416 (or 97%) and 313 (or 73%). Approval for data collection was obtained from the Central Committee on Research Involving Human Subjects in the Netherlands.

A trained interviewer visited the participants at home. In his or her presence all four family members individually completed the extensive questionnaire, which took about two hours. The family members were not allowed to discuss the questions with each other. When all four family members completed the questionnaire, the family received €30. Additionally, after three waves, five travel checks of €1,000 were raffled among the participating families. At T4, DNA samples were collected by means of saliva. A total of 311 unrelated adolescents agreed to be genotyped; five of these adolescents could not be genotyped. Attrition analyses were conducted to examine whether adolescents who gave their consent for genotyping (participants; n = 306) differed from the adolescents who did not (drop-outs; n = 122). T-tests showed no significant differences (p < .05) in loneliness, maternal support, sex, or age between participants and drop-out adolescents. Participating adolescents had slightly lower levels of education than those who were not included in the study (F(1,14) = -3.12, p = .002) and slightly higher levels of paternal support than drop-outs (F(4,25) = 2.15, p = .033).

**Measures**

**Loneliness.** The 12-item peer-related subscale of the Louvain Loneliness Scale for Children and Adolescents (LLCCA, Marceno et al., 1987) was completed at all time points. A sample item was ‘I feel abandoned by my friends’. All items were responded on a four-point scale (1 = never, 4 = often). Participants’ scores ranged from 12 to 48, with higher scores indicating higher levels of loneliness in the relationships with peers. Cronbach’s alpha ranged between .91 and .93 at the different measurement points. In previous studies high levels of internal consistency and moderate to high levels of construct validity were found for this scale (Marceno & Goossens, 1993, Marceno et al., 1987).

**Perceived support.** The adolescents completed a brief 12-item version of the Relational Support Inventory (RSI, Scholte, van Lieshout, & van Aiken, 2001) for support perceived from their fathers and mothers separately at T1. The items tapped several aspects of emotional support (e.g., ‘This person shows me that he/she loves me’) and instrumental support (e.g., ‘This person explains or shows how I can make or do something’). The participants rated each item on a 5-point Likert scale (1 = very untrue, 5 = very true) and the mean scores were computed for each parent separately. Total scores, therefore, ranged between 1 and 5. Cronbach’s alpha was .77 for maternal support, and .80 for paternal support.

**5-HTTLPR genotyping.** Genotyping of the HTTLPR polymorphism in the SLC6A4 (S-HTT, SERT) gene was performed by simple sequence length analysis. PCR was on 50 ng genomic DNA using 10 pmol of forward primer (5'-GGCGTGTGGCATCTGAAATGC-3') and 10 pmol reverse primer (5'-GAGGGACTGAGCTGGACAACCAC-3'), 25 mM dNTPs, 5 U Taq DNA polymerase (Invitro-gen, Breda, The Netherlands) in a PCR buffer containing 3 M Tris-HCl (pH 8.9), 75 mM ammoniumsulfate and 75 mM MgCl₂. The cycling conditions for the polymerase chain reaction started with 5 min at 92°C, followed by 35 cycles of 1 min at 92°C, 1 min at the optimized annealing temperature (57.5°C), and 1 min 72°C, then followed by an extra 5 min 72°C. PCR products were analyzed on a 2% agarose gel. The amplification yielded distinct bands at 484 bp (short ‘s’ allele) and 528 bp (long ‘l’ allele). To investigate the random genotyping error rate, the lab included 5 duplicate DNA samples per 96-well plate, which were 100% consistent. In addition, 4 blanks were included in each plate, which were required to be negative. By running PEDECHECK (O’Connell & Weeks, 1998) for single point Mendelian inconsistencies on the markers, we identified one family with potential pedigree errors. This family was removed from the analysis. Hardy–Weinberg equilibrium (HWE) proportions were estimated from parental genotype information using the Markov–Chain Monte–Carlo approximation of the exact test implemented in the GENEPOP package V 3.3 (Raymond & Rousset, 1995). No deviations from HWE were detected (p = .96). To maximize the power of the analyses, 5-HTTLPR genotype was dummy-coded into 1 (short-short and short-long) and 2 (long-long).

**Statistical Analyses**

We used latent growth curve modeling (LGCM) in Mplus (Muthen & Muthén, 1998–2007) to estimate both the initial level of loneliness at baseline (intercept) and the rate of change in loneliness from baseline across time (slope; Duncan, Duncan, & Strycker, 2006). As individual growth is estimated for each adolescent separately, LGCM is an excellent approach to take individual variations in the development of loneliness into account and to determine which predictors are associated with differential developments. Parameters in the models were estimated by applying the maximum likelihood estimator with robust standard errors (MLR), as required when dependent variables have non-normal distributions. In the first step, the basic model without the predictors was tested. In the second step, the relation between the 5-HTTLPR polymorphism and onset and development of loneliness was examined. In the third step, the relation between sex and
loneliness was examined and the interaction between sex and the 5-HTTLPR polymorphism was included in the model. All variables were centered before computing the interaction terms, to avoid multicollinearity. Finally, the relations between perceived support from fathers and mothers and loneliness were examined separately, and the interactions between perceived support and the 5-HTTLPR polymorphism (for fathers and mothers separately) were added to the model. Model fit was assessed by the following global fit indices: χ², CFI (with a cut-off value of .95) and RMSEA (with a cut-off value of .06) (Hu & Bentler, 1999).

Results

Descriptive Statistics

Of the 306 participants, 55 (18%) were homozygous for the short allele, 147 (48%) carried the heterozygous genotype, and 104 (34%) were homozygous for the long allele. The average level of loneliness at baseline (T1) was 18.85 (SD = 6.66). The mean levels of the five repeated measures of loneliness are illustrated in Figure 1. The level of loneliness marginally decreased over time (Wilks’ Λ = .97, F [4, 286] = 2.36, p = .053). The average scores for support were 4.11 (SD = 4.1) for perceived maternal support and 3.92 (SD = 4.7) for perceived paternal support. To check whether the levels of maternal support significantly differed from the levels of paternal support, we conducted a paired samples t-test, which showed that the levels of maternal support were significantly higher than the levels of paternal support, t(302) = 8.36, p < .001.

![Bar chart representing the mean levels of the repeated measures of loneliness.](image)

Figure 1 Bar chart representing the mean levels of the repeated measures of loneliness.

Correlations between 5-HTTLPR genotype, sex, support received from mother and father, and loneliness are depicted in Table 1. These findings showed that 5-HTTLPR genotype and sex were not significantly related to loneliness. Support from both mother and father were negatively related to loneliness at most time points.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
<th>7.</th>
<th>8.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 5-HTTLPR</td>
<td>-</td>
<td>2. Sex</td>
<td>.03</td>
<td>-</td>
<td>-.01</td>
<td>-</td>
<td>-.08</td>
<td>-</td>
</tr>
<tr>
<td>2. Support (mother)</td>
<td>.01</td>
<td>.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Support (father)</td>
<td>-.05</td>
<td>-.11</td>
<td>-.60</td>
<td>-.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. Loneliness (T1)</td>
<td>.02</td>
<td>-.08</td>
<td>-.18</td>
<td>-.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5. Loneliness (T2)</td>
<td>.02</td>
<td>-.06</td>
<td>-.07</td>
<td>-.15</td>
<td>.55</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. Loneliness (T3)</td>
<td>-.00</td>
<td>-.04</td>
<td>-.19</td>
<td>-.19</td>
<td>.51</td>
<td>.62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7. Loneliness (T4)</td>
<td>-.04</td>
<td>-.06</td>
<td>-.18</td>
<td>-.08</td>
<td>.42</td>
<td>.47</td>
<td>.53</td>
<td>-</td>
</tr>
<tr>
<td>8. Loneliness (T5)</td>
<td>-.11</td>
<td>-.10</td>
<td>-.15</td>
<td>-.03</td>
<td>.48</td>
<td>.38</td>
<td>.47</td>
<td>.70</td>
</tr>
</tbody>
</table>

Note: * p < .05, ** p < .01.

Model Findings

First, the basic model without predictors was tested. The intercept and slope were significant (β₁ = 18.939, p < .001; β₂ = -.334, p < .010), which indicated that participants on average scored 18.93 on the loneliness scale at baseline, and the level of loneliness decreased over time (β₃ [df = 10, n = 304] = 345.75, CFI = .92, and RMSEA = .095). Second, we included the 5-HTTLPR genotype as a predictor in the model (β₄ [df = 13, n = 304] = 40.58, CFI = .93, and RMSEA = .084). The relation between the 5-HTTLPR genotype and the intercept was not significant (β = .057, SE = .068, p = .402). The 5-HTTLPR genotype was significantly related to the slope (β = -1.69, SE = .082, p = .041). The relation between the 5-HTTLPR genotype and the slope of loneliness is illustrated in Figure 2. As can be seen in Figure 2, carriers of the long-long genotype decreased in loneliness over time, while the levels of loneliness in participants carrying at least one short allele remained relatively stable over time.

Third, the relation between sex and the level of loneliness was examined. Sex was included as a predictor, along with the 5-HTTLPR genotype. The 5-HTTLPR genotype was still negatively related to the slope, whereas sex was positively related to the slope (β = .201, SE = .080, p = .012), indicating that the level of loneliness in girls generally remained stable over time, while the level of loneliness in boys slightly decreased. In addition, the interaction term between sex and the 5-HTTLPR genotype was included as a predictor in

![Figure 1](image)
The aim of the present study was to examine the effect of 5-HTTLPR genotype, sex, parental support, and their interactions on the onset and development of loneliness in adolescence. Our longitudinal results, the first to appear in the published literature across such a relatively long period, showed that the levels of loneliness were highest in early adolescence and slowly decreased throughout adolescence. This is in accordance with our expectations and an earlier cohort study (Marcoen & Goossens, 1993).

We did not find any effects of 5-HTTLPR genotypes on baseline levels of loneliness. These results were in line with cross-sectional research on the relations between 5-HTTLPR genotype and depression, in which direct effects of the 5-HTTLPR genotype were rarely found (for a review, see Munafo et al., 2009). A prominent finding of our study, however, was the genetic underpinning of changes in loneliness in the teenage years: the 5-HTTLPR genotype was related to the development of loneliness, with short allele carriers remaining stable in loneliness over time, whereas adolescents with the long-long genotype showed a decrease in loneliness. Our results can be interpreted in different ways. First, both loneliness and depressive symptoms reflect a high degree of negative affectivity (i.e., a high tendency to develop negative feelings). Short allele carriers have been found to be at risk for developing problems with negative emotion regulation (e.g., Pezawas et al., 2005), that are also implied in the development of loneliness or, as our results indicated, to being unable to reduce feelings of loneliness. Second, loneliness is a precursor (Qualter, Brown, Munn, & Rotenberg, 2010) or even a proxy for depressive symptoms. Future research on the underlying mechanism is in order.

The model fitted the data relatively well ($\chi^2$ [df = 19, n = 304] = 52.03, CFI = .93, and RMSEA = .076), but the interaction term was neither related to intercept nor slope.

In the fourth model, the associations between support from father and mother and loneliness were examined. First, both support from father and the 5-HTTLPR genotype were included as predictors in the model. Paternal support was negatively related to the intercept ($\beta = -2.69, p = .000$) and positively to the slope ($\beta = .215, p = .041$), whereas the 5-HTTLPR genotype was significantly related to neither the intercept nor the slope ($p = .500$ and .051, respectively). This model showed a relatively good fit ($\chi^2$ [df = 16, n = 304] = 44.47, CFI = .93, and RMSEA = .077). The interaction term between support from father and the 5-HTTLPR genotype was included in the model. This interaction was not significantly related to either the intercept or the slope (respectively $\beta = -.051, p = .807, \beta = -.061, p = .830$). Support from mother was negatively related to the intercept ($\beta = -.196, p = .003$), but not to the slope. The 5-HTTLPR genotype was not significant in predicting the intercept, but predicted the slope of loneliness ($\beta = -.169, p = .040$). Subsequently, the interaction term between support from mother and the 5-HTTLPR genotype was included in the model, which is illustrated in Figure 3. This interaction was positively related to the intercept of loneliness ($\beta = .564, p = .003$), indicating that short allele carriers who received high social support from their mothers had lower levels of loneliness at baseline than short allele carriers who received low support. For the long-long genotype, no significant relation existed between support and the intercept of loneliness ($\chi^2$ [df = 19, n = 304] = 54.23, CFI = .92, and RMSEA = .078).

The development of loneliness for the different 5-HTTLPR genotypes is illustrated in Figure 2. Maternal support and the 5-HTTLPR genotype were included in the model ($\chi^2$ [df = 16, n = 304] = 49.08, CFI = .92, and RMSEA = .082). Support from mother was negatively related to the intercept ($\beta = -.196, p = .003$), but not to the slope. The 5-HTTLPR genotype was not significant in predicting the intercept, but predicted the slope of loneliness ($\beta = -.169, p = .040$). Subsequently, the interaction term between support from mother and the 5-HTTLPR genotype was included in the model, which is illustrated in Figure 3. This interaction was positively related to the intercept of loneliness ($\beta = .564, p = .003$), indicating that short allele carriers who received high social support from their mothers had lower levels of loneliness at baseline than short allele carriers who received low support. For the long-long genotype, no significant relation existed between support and the intercept of loneliness ($\chi^2$ [df = 19, n = 304] = 54.23, CFI = .92, and RMSEA = .078).

Figure 2 The development of loneliness for the different 5-HTTLPR genotypes.

Figure 3 Interactions between 5-HTTLPR genotype and maternal support.

The aim of the present study was to examine the effect of 5-HTTLPR genotype, sex, parental support, and their interactions on the onset and development of loneliness in adolescence. Our longitudinal results, the first to appear in the published literature across such a relatively long period, showed that the levels of loneliness were highest in early adolescence and slowly decreased throughout adolescence. This is in accordance with our expectations and an earlier cohort study (Marcoen & Goossens, 1993).

We did not find any effects of 5-HTTLPR genotypes on baseline levels of loneliness. These results were in line with cross-sectional research on the relations between 5-HTTLPR genotype and depression, in which direct effects of the 5-HTTLPR genotype were rarely found (for a review, see Munafo et al., 2009). A prominent finding of our study, however, was the genetic underpinning of changes in loneliness in the teenage years: the 5-HTTLPR genotype was related to the development of loneliness, with short allele carriers remaining stable in loneliness over time, whereas adolescents with the long-long genotype showed a decrease in loneliness. Our results can be interpreted in different ways. First, both loneliness and depressive symptoms reflect a high degree of negative affectivity (i.e., a high tendency to develop negative feelings). Short allele carriers have been found to be at risk for developing problems with negative emotion regulation (e.g., Pezawas et al., 2005), that are also implied in the development of loneliness or, as our results indicated, to being unable to reduce feelings of loneliness. Second, loneliness is a precursor (Qualter, Brown, Munn, & Rotenberg, 2010) or even a proxy for depressive symptoms. Future research on the underlying mechanism is in order.
Additionally, a main effect of sex on the development of loneliness was found, with girls being stable in loneliness over time, and with boys decreasing. Because the origins of these differences remain unclear, sex differences in loneliness should be examined in greater detail in future studies. The interaction between 5-HTTLPR genotype and sex was not significant, which implies that sex does not have a moderating role in the relation of 5-HTTLPR genotype and loneliness. This finding contrasts with the results of an earlier cross-sectional study on depression in adolescence, in which a direct effect of 5-HTTLPR genotype was found exclusively for girls (Eley et al., 2004). Perceived parental support was found to be pivotal in the onset and the development of loneliness. In line with our expectations, perceived support from both father and mother were negatively related to the baseline level of loneliness, indicating that high levels of perceived parental support can be seen as a protective factor against loneliness. Support received from mother was not related to the development of loneliness over time, whereas support from father was positively related to the slope of loneliness, which implies that high levels of paternal support lead to an increase in loneliness. A possible explanation is that fathers might react to the emotional problems of their child at baseline by providing more support, which has been found in a previous study (Eisenberg, Fabes, & Murphy, 1996). A possible reason why we did not find this association in mothers is that, in our sample, mothers scored significantly higher on support than fathers. The gene–environment interaction between paternal support and 5-HTTLPR genotype was not significant, whereas the interaction between maternal support and 5-HTTLPR genotype was significantly related to the intercept of loneliness. These results show that adolescents who received little support from their mother and carried a short allele were at increased risk for developing loneliness and that they might benefit more from higher levels of maternal support. This is in line with results from a study by Fox et al. (2005), who found that the 5-HTTLPR genotype interacts with maternal support in predicting children’s behavioral inhibition and shyness. Although no studies yet have examined the role of 5-HTTLPR genotype in loneliness, studies on depression showed that this genotype often interacted with parental behavior, such as support, in predicting depression in children and adolescents (Kauffman et al., 2004; Sjöberg et al., 2006). These results are also in line with the presumed underlying mechanism in depression, in which short allele carriers have problems with regulation of negative emotions. Carrying a short allele can put one at risk, but the problematic behavior may only come to the fore when another risk factor, such as low maternal support, is present.

Since loneliness is a complex phenotype, it is likely that multiple environmental and biological factors influence its onset and developmental course. Regarding the environment, environmental assets and stressors other than perceived parental support may exert their effects on adolescent loneliness as well. For example, stressful life events have been found to be important in loneliness (Segrin, 1999) and to interact with the 5-HTTLPR genotype in predicting depression (Caspi et al., 2003). Concerning genetic vulnerabilities, additional polymorphisms involved in the serotonin pathway may also play a role in loneliness. Polymorphisms related to the enzymes tryptophan hydroxylase 1 and 2, which are involved in serotonin synthesis, have been related to depression (Gizatullin, Zaboli, Jönsson, Asberg, & Leopardi, 2006) and are involved in the basic emotion regulation circuit (Brown et al., 2005). In future studies with large longitudinal samples, it is important that multiple environmental and genetic influences on loneliness will be examined. Finally, it is important to recognize that environmental factors have direct effects, and in addition show moderate heritability and specific associations with genes. Studies on depression revealed that exposure to negative life events is partly genetically determined (Paykel, 2003) and that key features of negative parenting, such as low parental support, are also partly genetically determined. Such additional effects of environmental factors on loneliness should be considered in future research.

Limitations

A number of limitations have to be mentioned. First, like any findings on gene–environment interactions in non-clinical samples, our results are primarily hypothesis-generating or exploratory. The next step is to establish clinical relevance of the gene–environment interaction obtained within a clinical population, which includes the estimation of the strength of the interaction (Dempfle et al., 2008). Second, population stratification (Marchini, Cardon, Phillips, & Donnelly, 2004) might have occurred, although this is not very likely because the number of adolescents not born in the Netherlands was very low (1.2%), and the number of adolescents not born in a European country was even lower (0.2%). Further, the 5-HTTLPR genotype frequencies were in line with the frequencies usually found in Caucasian samples (Harrir & Holmes, 2006), which minimizes the chance that population stratification occurred. Third, support from parents was reported by the adolescents, which may not be a precise measure of the actual behavior. However, the way adolescents perceived the support given may be more important than the actual support provided by parents (Steinberg, Lamborn, Dornbusch, & Darling, 1992).

Conclusion

Our study is the first to explore adolescent loneliness longitudinally and to examine the genetic underpinnings of loneliness. Our results contribute to a further understanding of the environmental and genetic bases of loneliness. Our main finding was that the 5-HTTLPR genotype was related to the development of loneliness, and interacted with maternal support in predicting the level of loneliness at baseline. We would like to emphasize that replication of our results is needed in both population-based and clinical samples with sufficient sample size, before any recommendations for interventions can be made.
Chapter 8

The dopamine D2 receptor gene, perceived parental support, and adolescent loneliness: Longitudinal evidence for gene-environment interactions

Published as:
Abstract

Loneliness is a common problem in adolescence. Earlier research focused on genes within the serotonin and oxytocin systems, but no studies have examined the role of dopamine-related genes in loneliness. In the present study, we focused on the dopamine D2 receptor gene (DRD2). Associations among the dopamine D2 receptor gene (DRD2), sex, parental support, and loneliness were examined in a longitudinal study spanning five annual waves (N = 307). Using Latent Growth Curve Modeling (LGCM), DRD2 genotype was not directly related to loneliness. Interactions were found between parental support and DRD2 genotype, showing that adolescents with the A2A2 genotype who perceived little support from their parents had the highest baseline levels of loneliness. Adolescents with an A1 allele were not susceptible to the rewarding effect of parental support. The present study is the first to examine the role of the DRD2 genotype in loneliness. Our results contribute to a further understanding of the environmental and genetic basis of loneliness in adolescence.

Introduction

Loneliness is a common experience that can be present in all phases of life, particularly in adolescence (Heinrich & Gullone, 2006). This experience is typically defined as the negative emotional response to a discrepancy between the desired and achieved quality and quantity of one’s social network (Perlman & Peplau, 1981). People can feel lonely because they have fewer friends compared to others or they can have a sufficient number of friends but feel dissatisfied with the overall quality of their relationships with these friends. Chronic loneliness has been related to Diagnostic and Statistical Manual of Mental Disorders (DSM)-based major depressive disorder, anxiety disorders, and schizophrenia (Heinrich & Gullone, 2006) and to physical health problems (e.g., cardiovascular disease, poorer immune functioning, Cacioppo et al., 2002). Therefore, it is important to examine its antecedents. The aim of the present study is to examine the predictors of loneliness in adolescence, with a focus on genetic factors (DRD2 genotype) and parental support.

Behavioral genetic studies have shown that loneliness is moderately heritable, with heritability estimates ranging between 48% and 55% (Boomsma et al., 2005). Up to now, however, only two molecular genetic studies have examined associations between loneliness and specific genes (Lucht et al., 2009; van Roekel, Scholte, et al., 2010). Lucht and colleagues (2009) found a relation between the oxytocin receptor gene (OXTR) and emotional loneliness in adults, whereas Van Roekel et al. (2010) found a significant association between the serotonin transporter gene (5-HTTLPR) and the development of loneliness throughout adolescence. Although these studies provide interesting insights, additional genes could be involved in loneliness as well.

A recent theory on loneliness states that lonely people do not enjoy positive events as much as non-lonely individuals do (Hawkley & Cacioppo, 2010). For example, a diary study has shown that individuals high on loneliness experienced less reward from daily social interactions compared to individuals low on loneliness (Hawkley et al., 2007). Experiences of reward and enjoyment are typically associated with the dopamine system in the brain (e.g., Blum et al., 1996). A Functional Magnetic Resonance Imaging (fMRI) study on loneliness found that lonely people showed less activation in the ventral striatum in response to positive social stimuli compared to non-lonely people (Cacioppo et al., 2009). Within this system, the dopamine receptor D2, which is regulated by the DRD2 gene, is of interest (Blum et al., 1996). The A1 allele of the DRD2 gene was found to be associated with reduced dopamine D2 receptor binding in the ventral striatum, a region of the brain that is involved in reward mechanisms (Thompson et al., 1997). It is assumed that this reduced D2 receptor binding leads to experiencing less reward (Blum et al., 1996). This was also shown in an fMRI study (Cohen et al., 2005) in which participants carrying at least one A1 allele showed lower responses in brain areas involved in reward mechanisms (e.g., nucleus accumbens) when presented with a reward. These findings indicate that people carrying at least one A1 allele have reduced binding of DRD2 receptors in the brain reward system.
and are less sensitive to rewards compared to people homozygous for the A2 allele. As lonely people showed lower levels of reward in response to positive social stimuli, this might suggest lower D2 binding capacities in certain brain areas. Therefore, we expected that the DRD2 genotype would be related to loneliness.

To our knowledge, the existing research has not examined direct effects of the DRD2 gene on loneliness; however, mixed findings were obtained regarding depression, a condition related to loneliness. Some studies (Audrain-McGovern, Lerman, Wileyto, Rodriguez, & Shields, 2004; Guo & Tillman, 2009; Lawford, Young, Noble, Kann, & Ritchie, 2006) found that A1 carriers evidenced more depressive symptoms, but this effect was not confirmed in other studies (e.g., Elovainio et al., 2007). Such discrepant findings are common in molecular genetic studies (e.g., Lin, Vance, Pericak-Vance, & Martin, 2007).

Most studies on the determinants of loneliness in adolescence have focused on environmental factors. A meta-analysis (Mahon et al., 2006) revealed that the most important environmental predictors of loneliness were social support and both maternal and paternal expressiveness. More specifically, several studies find that high levels of both maternal and paternal support prevent feelings of loneliness in adolescence (Franzoi & Davis, 1985; Mounts et al., 2006). Several explanations are offered for this association. High levels of parental support indicate an overall closer parent-adolescent relationship in general and higher levels of communication, allowing adolescents to talk about their feelings of loneliness with their parents (Franzoi & Davis, 1986; Mounts et al., 2006). Next to direct environmental influences, direct gene effects on multi-factorial forms of psychopathology, such as depression, anxiety and loneliness (Rutter et al., 1997), are often small and hence difficult to detect. In addition, the effects of certain genes may only be expressed under particular environmental conditions, most often exposure to negative environments (Rutter, 2007). Until now, most studies have examined interactions between genes and negative environments. Studies examining interactions between stress and the DRD2 genotype have revealed inconsistent results. One study found that individuals with the (higher activity) A2A2 genotype were more affected by environmental stress, and as a consequence had higher levels of depression (Elovainio et al., 2007), whereas another study (Vaske, Beaver, Wright, Boisvert, & Makarios, 2009) found A1 carriers to be more depressed when they experienced environmental stress.

However, no studies on gene-environment interactions have reported on positive environmental influences, such as parental support. Other positive environmental variables, such as social cooperative behavior and social comparison, were also associated with activation in the ventral striatum (Fliessbach et al., 2007; Rilling et al., 2002). Because A1 allele carriers have fewer D2 receptors in the ventral striatum (Thompson et al., 1997) and show lower responses to rewarding stimuli (Cohen et al., 2005), they may be less sensitive to these social types of reward as well. Therefore, we hypothesized that A1 carriers would be less susceptible to parental support, and that adolescents with the A2A2 genotype would benefit from higher levels of support.

The aim of our study was to examine the relationships of the DRD2 genotype, parental support, and DRD2 by parental support interactions with adolescent loneliness. We used a longitudinal five-wave design, which allowed us to examine relations with both the baseline level of loneliness and the development over time. Because previous studies found differences between boys and girls in the level of loneliness (e.g., Koening & Gladstone, 1998; van Roekel, Scholte, et al., 2010), we also examined sex differences. We did not have a specific hypothesis for the relation between DRD2 genotype and loneliness because the results from studies examining relations between DRD2 genotype and depression are inconsistent. For the gene-environment interactions, we expected that parental support would not affect A1 carriers, which would result in a non-significant relation between support and loneliness in this group. However, adolescents homozygous for the A2 allele would be susceptible to positive social rewards; therefore, they may benefit from higher levels of support. Hence, we expected that high levels of parental support would be related to lower levels of loneliness in adolescents with the A2A2 genotype.

Method

Procedure

Data for the present study were derived from a longitudinal Dutch survey study called Family and Health with five annual waves which examined different socialization processes in relation to various health behaviors among adolescents and their families (Harakeh et al., 2005). For a detailed description of the procedure, see Van der Zwaluw et al. (2008).

Participants

The present study used data from the youngest adolescents in each family. These adolescents were entering adolescence at T1, which allowed us to examine loneliness throughout adolescence. Our final sample consisted of 307 adolescents, of which 53.4% were girls. The mean age at T1 was 13.4 years (SD = 5.1). One-third (33.4%) of the adolescents attended lower education (i.e., preparatory secondary school for technical and vocational training), one-third (36.8%) intermediate general education (i.e., preparatory secondary school for college), and one-third (29.8%) attended the highest level of secondary school (i.e., preparatory secondary school for university). A small group of adolescents were not born in the Netherlands (1.2%), and of this group, 0.2% were not born in a European country.

Attrition analyses were conducted to examine whether adolescents who gave their consent for genotyping (participants; n = 307) differed from the adolescents who did not (dropouts; n = 121). T-tests showed no significant differences between participants and dropouts (p > .05) in loneliness, maternal support, or age. However, participants did
experience a slightly lower level of paternal support compared to dropouts (0[426] = 2.164, \( p = .03 \)). For educational level and sex, Chi squared statistics were calculated to examine differences in education and sex between dropouts and participants. Significant differences existed for educational level, indicating that participating adolescents had a higher level of education compared to dropouts (\( \chi^2[416] = 7.61, p = .02 \)). No sex differences were found in retention (\( \chi^2[428] = 0.50, p = .48 \)).

**Measures**

**Loneliness.** Loneliness was measured at five time points using 12 items from the peer-related subscale of the Louvain Loneliness Scale for Children and Adolescents (LLCA; Marcoen et al., 1987). Sample items were, ‘I feel abandoned by my friends’ and ‘I feel sad because I have no friends.’ The items were measured on a four-point scale ranging from (1) never to (4) always. Scores ranged from 12 to 48, with higher scores indicating higher levels of loneliness. Cronbach’s alpha ranged from .91 to .93 at different time points.

**Perceived parental support.** Participants completed a 12-item version of the Relational Support Inventory (RSI; Scholte et al., 2001) at T1. The scale measures aspects of emotional and instrumental support. Participants completed the questionnaire for fathers and mothers separately. The questionnaires for maternal and paternal support were identical. Example items are: ‘My mother/father supports me in the things I do;’ and ‘My mother/father explains or shows how I can make or do something.’ Each item was rated on a 5-point Likert scale ranging from (1) very untrue to (5) very true. Alpha was .77 for maternal support, and .80 for paternal support.

**DRD2 genotyping.** The DRD2 TaqI A C>T polymorphism was genotyped using Taqman analysis (assay ID: Taqman assay:C___7486676_10; reporter 1: VIC -A-allel, reverse assay; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 10 ul containing 10 ng of genomic DNA, 5 ul of Taqman Mastermix (2x; Applied Biosystems), 0.125 ul of the Taqman assay, and 3.875 ul of H2O. Genotyping was performed on a 7500 Fast Real-Time PCR System and genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems).

To investigate the random genotyping error rate, the lab included 5 duplicate DNA samples per 96-well plate, which were 100% consistent. In addition, 4 blanks were included in each plate, which were required to be negative. By running PEDCHECK (O’Connell & Weeks, 1998) for single point Mendelian inconsistencies on the markers, we identified one family with potential pedigree errors. This family was removed from the analysis. Hardy–Weinberg equilibrium (HWE) proportions were estimated from parental genotype information using the Markov–Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3 (Raymond & Rousset, 1995). No deviations from HWE were detected (\( p = .96 \)). To maximize the power of the analyses, DRD2 genotype was dummy-coded into 0 (A2A2) and 1 (A1A2 and A1A1).

**Statistical Analyses**

Latent Growth Curve Modeling (LGCM) was used to estimate both the individual level of loneliness at baseline (i.e., intercept), and the change in loneliness over time (i.e., slope, Duncan et al., 2006). In this approach, it is not assumed that all participants start at the same level of loneliness at baseline and have the same rate of change in loneliness over time, instead, individual growth is examined for each participant. Therefore, LGCM is an excellent way to examine individual variation in the development of loneliness and to investigate whether certain predictors relate to these changes over time. Mplus (Muthén & Muthén, 1998-2007), a statistical software program designed for Structural Equation Modeling (SEM) analyses, was used for these analyses. Parameters in the models were estimated by applying a method that corrects for the non-normal distribution of the dependent variables. This method is referred to as the maximum likelihood estimator with robust standard errors or MLR. To deal with missing data, which were rare, we did not impute these data but borrowed information from the observed portion of the data. This approach (which is referred to as the full-information maximum likelihood or FIML approach) is superior to other techniques for handling missing data, such as multiple imputation, pairwise deletion, or listwise deletion (Muthén & Muthén, 1998-2007).

First, we tested the initial developmental model, estimating the intercept (or initial level) and slope (or rate of change) in loneliness regardless of genetic or environmental influences. Second, we examined the relation between the DRD2 genotype and the initial or baseline level and rate of change in loneliness (Model 2). Third, we examined the main effects of maternal and paternal support on loneliness (Models 3 and 4). Finally, we examined the interactions between parental support and the DRD2 genotype (Models 5 and 6). To avoid multi-collinearity, all variables were centered before computing the interaction terms. Model fit was assessed by the following global fit indices: \( \chi^2 \) (with a cut-off value of .05) and Root Mean Square Error of Approximation (RMSEA) (with a cut-off value of .06; Hu & Bentler, 1999).

**Results**

**Descriptive Statistics**

Of the 307 participants, 205 (66.3 %) were homozygous for the A2 allele, 96 (31.1 %) had the heterozygous genotype, and 8 (2.6 %) were homozygous for the A1 allele. Table 1 shows means, standard deviations, and Pearson correlations among model variables. The average level of loneliness across the five time points was relatively low in absolute terms, with means ranging from 17.67 to 18.81 (out of a maximum score of 48). These means were comparable with other studies on community samples (Marcoen & Goossens, 1993). The loneliness scores ranged from 12 - 48 at T1, 12 - 45 at T2, 12 - 38 at T3, 12 - 46 at T4, and 12 - 44 at T5. Sex was dummy-coded (0 = boys, 1 = girls) so that the average score for sex in
Second, the DRD2 genotype was included as a predictor in the model, but it was not significantly related to intercept or slope. To examine whether the intercept and slope differed for boys and girls, we included sex as a predictor. The results showed a significant relation between sex and the slope, indicating that girls remained relatively stable in loneliness over time, whereas loneliness decreased among boys. No relation was found between sex and baseline levels of loneliness. Because of the sex differences in the slope of loneliness, we tested whether the DRD2 genotype explained this relation by adding the interaction between sex and the DRD2 genotype to the model. This interaction was not related to the intercept of loneliness or the slope. Third, we examined relations between parental support and loneliness. We examined direct relations between parental support and loneliness first while controlling for sex. Both maternal and paternal support were negatively related to the intercept but not to the slope. Low levels of support were related to high levels of loneliness at baseline. In the next step, we examined the interactions between parental support and the DRD2 genotype for maternal and paternal support separately. For both maternal and paternal support, the interaction was significantly related to the intercept of loneliness, as depicted in Figure 1. (Because the figures were similar for paternal and maternal support, only the figure for maternal support is shown). No relation existed between parental support and baseline levels of loneliness for adolescents who carry at least one A1 allele, whereas low parental support in adolescents with the A2A2 genotype was related to higher levels of loneliness at baseline.

Table 1 reflects the proportion of girls in the sample. Concurrent correlations at T1 showed a significant negative association between loneliness and parental support, as expected. Support from both father and mother were negatively related to loneliness at most waves. The correlations showed that the DRD2 genotype and sex did not relate to loneliness. No sex differences were found for any of the measures.

Model Findings
First, we tested the initial developmental model (i.e., no predictors, Table 2). The intercept and slope were significant, indicating that participants scored, on average, 18.88 on loneliness at baseline and that the level of loneliness decreased significantly over time.

Table 2 Regression of Initial Level (Intercept) and Rate of Change (Slope) in Adolescents’ Loneliness on Gene x Environment Interactions

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Intercept</th>
<th>Slope</th>
<th>χ² (df)</th>
<th>CFI</th>
<th>RMSEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initial developmental model</td>
<td>18.88 (0.35)**</td>
<td>-0.29 (0.10)*</td>
<td>37.81 (10)</td>
<td>92</td>
<td>0.095</td>
</tr>
<tr>
<td>2. DRD2</td>
<td>-0.03 (0.07)</td>
<td>0.11 (0.08)</td>
<td>40.50 (13)</td>
<td>93</td>
<td>0.083</td>
</tr>
<tr>
<td>3. Sex</td>
<td>-0.04 (0.07)</td>
<td>0.19 (0.08)*</td>
<td>48.13 (16)</td>
<td>93</td>
<td>0.081</td>
</tr>
<tr>
<td>4. DRD2 x Sex</td>
<td>0.49 (0.28)</td>
<td>-0.24 (0.32)</td>
<td>55.07 (19)</td>
<td>92</td>
<td>0.079</td>
</tr>
<tr>
<td>5. Maternal support</td>
<td>-0.18 (0.07)**</td>
<td>-0.05 (0.10)</td>
<td>52.79 (16)</td>
<td>92</td>
<td>0.087</td>
</tr>
<tr>
<td>6. DRD2 x Maternal support</td>
<td>0.67 (0.17)**</td>
<td>-0.65 (0.28)*</td>
<td>55.66 (22)</td>
<td>93</td>
<td>0.071</td>
</tr>
<tr>
<td>7. Paternal support</td>
<td>-0.25 (0.07)**</td>
<td>0.17 (0.10)</td>
<td>48.48 (16)</td>
<td>92</td>
<td>0.081</td>
</tr>
<tr>
<td>8. DRD2 x Paternal support</td>
<td>0.62 (0.19)**</td>
<td>-0.75 (0.26)**</td>
<td>51.31 (22)</td>
<td>94</td>
<td>0.066</td>
</tr>
</tbody>
</table>

Note. DRD2 = Dopamine D2 receptor gene. * p < .05. ** p < .01. *** p < .001.
In all analyses, we controlled for sex. Only new variables entered in the model are depicted in the table.

Figure 1 Interactions between DRD2 genotype and maternal support on intercept of loneliness.
The interaction was also significantly related to the slope of loneliness, for both maternal and paternal support. Parental support did not relate to the slope of loneliness in adolescents carrying at least one A1 allele. However, in adolescents with the A2A2 genotype, lower levels of parental support were related to a faster decrease in loneliness over time. As seen in Figure 2 (paternal support, the results were similar for maternal support), this effect was due partly to the high levels of loneliness at baseline for adolescents who experienced low levels of support. These adolescents started with the highest levels of support at baseline, subsequently decreased at a faster rate, but still ended up with higher levels of loneliness at T5 compared to adolescents who experienced medium or high levels of support. Finally, we conducted multigroup analyses to examine sex differences. These analyses showed no differences in effects between boys and girls.

**Figure 2** Development of loneliness for A2A2 genotype, split by paternal support.

**Discussion**

The aim of the present study was to examine the relations between DRD2 genotype, parental support, and loneliness in adolescence. We did not find a direct relation between DRD2 genotype and the baseline and rate of change in loneliness, which is in line with our hypothesis. We did find that boys decreased in loneliness at a faster rate than girls did, but the DRD2 genotype could not explain this relation as the interaction between sex and the DRD2 genotype was not significant. One possible explanation for the non-significant direct effects of DRD2 genotype in our sample is that direct relations between genes and complex mental problems are often small and therefore difficult to detect (Rutter, Moffitt, & Caspi, 2006).

Both paternal and maternal support were negatively related to the baseline level of loneliness. Adolescents who experienced low levels of support had the highest levels of loneliness at baseline. This was in line with our hypothesis and previous research (e.g., Franzoi & Davis, 1985; Mounts et al., 2006). However, the results from the gene-environment interactions showed that this relation only holds for adolescents carrying the A2A2 genotype, as expected. In adolescents with the A2A2 genotype, a negative relation was found, in that adolescents who experienced low levels of support showed the highest levels of loneliness at baseline. Adolescents with an A1 allele were not affected by support from their father or from their mother. This is an important finding because it implies that adolescents with the A2A2 genotype may benefit from high levels of social support. It may be that these adolescents experience adequate levels of social reward when interacting with their parents, whereas adolescents with A1 genotypes run a risk for internalizing problems due to inadequate levels of social reward experienced in general. This lack of reward experience in A1 allele carriers may be caused by the reduced concentration of D2 receptors in the brain areas associated with reward (Thompson et al., 1997).

In addition to the relations with the intercept, adolescents with the A2A2 genotype who experienced the lowest levels of support, decreased in loneliness at a faster rate compared to adolescents experiencing high levels of support. At first glance, this is in contrast with our expectations. However, Figure 2 shows that this result is due mainly to the negative effect of parental support on the baseline level of loneliness. A possible explanation for these results may be that adolescents who receive low levels of parental support try to compensate for this by seeking more support from other people, such as peers or friends (Scholte et al., 2001). Therefore, their loneliness levels may decrease at a faster rate but remain at high levels because parental support is still an important predictor of loneliness.

**Theoretical Implications**

The results from the present study, combined with the results from previous molecular genetic studies on loneliness (Lucht et al., 2009; Van Roekel et al., 2010), provide important insights into the biological and genetic underpinnings of loneliness. In addition to these studies, a recent theory (Hawkley & Cacioppo, 2010) states that people who score high on loneliness (a) do not enjoy positive events as much as do non-lonely individuals and (b) show a hypervigilance for social threats. These combined characteristics lead to a self-reinforcing loop of increasingly negative social expectations that ultimately give rise to the negative sequelae of sustained loneliness.
Experiences of reward and enjoyment are typically associated with the dopamine system in the brain and the dopamine receptor D2 gene. As our results show, individuals who carry at least one A1 allele are not susceptible to a particular type of social reward, that is, parental support. This may indicate that they also experience less joy from positive events, which is one of the characteristics of lonely people proposed in the former theory (Hawkley & Cacioppo, 2010). In addition, research on oxytocin has shown that increased levels of this neuropeptide are related to attachment, bonding, and reward dependence (Bora, Yucel, & Allen, 2009). This may indicate that oxytocin also plays a role in the lowered social reward experiences in lonely people, which may explain why a relation was found between the oxytocin receptor gene and emotional loneliness in adults (Lucht et al., 2009). Hypervigilance to threat is often associated with the serotonin system in the brain and, in particular, with peculiarities regarding the serotonin transporter, as regulated by the serotonin transporter gene (5-HTTLPR). Individuals who carry at least one copy of the short allele of this gene show an increased risk for hypervigilance to threat (Pezawas et al., 2005). More research on the specific mechanisms underlying the relations between these three genes and loneliness is warranted.

**Clinical Implications**

The findings on gene-environment interactions in the current study may also inform intervention efforts for lonely people. As loneliness is jointly determined by genetic and environmental influences, it is vital for counselors and therapists to take into account the degree of perceived parental support. Adolescents who enjoy high levels of support experience lower levels of loneliness, at least when their capacity to enjoy social contacts is unimpaired. This additional source of support may be put to good effect in the actual interventions developed for lonely people.

Research on the genetic underpinnings of social cognition in lonely people may have clinical implications for interventions designed to reduce loneliness. A recent review of such efforts revealed that interventions that directly tackled the way in which lonely people think about social situations were more successful compared to other types of interventions (Masi, Chen, Hawkley, & Cacioppo, 2011). Pending further research on the model previously described (Hawkley & Cacioppo, 2010), interventions may try to both decrease hypervigilance to social threat and teach lonely people to enjoy social contacts with other people more fully.

**Limitations and Suggestions**

This study has some limitations that should be addressed. First, our sample scored relatively low on loneliness. This could be due to the inclusion criteria, which required that parents be married or living together. Moreover, participating family members had to be biologically related to each other. Because of these restrictions, our sample may have comprised relatively well-functioning families. Future research should focus on testing relations between DRD2 genotype and loneliness in clinical samples or at-risk groups in which support levels may be lower (Thomson, Hanson, & McLeanahan, 1994). Second, we only examined parental support as an environmental influence, whereas other variables may also be important. For example, research has shown that peer-related variables, such as peer support (Domiitrovich & Bierman, 2001) and self-disclosure to peers (Franzo & Davis, 1965), play a role in adolescent loneliness. Future studies could examine those factors in relation with the DRD2 genotype and loneliness. In addition, genetic factors may also influence parental support, as previous research on parenting has shown (e.g., van IJzendoorn, Bakermans-Kranenburg, & Mesman, 2008). However, we did not find a correlation between parental support and the adolescents’ genotype; therefore, we may conclude that this has not influenced our analyses. Third, the participating adolescents completed both the loneliness and parental support measures. Correlations between these measures, therefore, may partially reflect shared method variance. Still, it has been suggested that the adolescents’ perception of the support they receive may be more important than the actual support that parents provide (Steinberg et al., 1992). Fourth, there are some inherent problems associated with gene-environment interaction research. Typically, this type of interaction is difficult to replicate, and replications of an initially positive result always require larger samples than used in the original positive study (Rutter et al., 2006). Therefore, it is important to emphasize that replication of the findings of the present study is needed in both population-based and clinical samples.

Another suggestion for further research is to examine the effects of DRD2 genotype on responses in reward brain areas in lonely people, because the ventral striatum might be implicated in loneliness (Cacioppo et al., 2009) and this brain area is an important site of D2 receptor density (Thompson et al., 1997).

**Conclusion**

Although we did not find a direct effect of the DRD2 genotype on loneliness, an important finding of our study was that adolescents homozygous for the A2 allele were more susceptible to social rewards and therefore experienced less loneliness when they received high levels of parental support. It is important to stress that replication of these findings is needed in both population-based and clinical samples.
Chapter 9

Oxytocin receptor gene (OXTR) in relation to loneliness in adolescence: Interactions with sex, parental support, and DRD2 and 5-HTTLPR genotypes

Published as:
**Abstract**

Recent research revealed that loneliness, a common problem in adolescence, may have a genetic basis. The evidence, though, was limited mostly to serotonin- and dopamine-related genes. In the present study, we concentrated on the oxytocin receptor gene (OXTR). Associations were examined in a longitudinal study spanning five annual waves (N = 307). Relations between OXTR and loneliness were examined, as well as interactions between OXTR and sex, parental support, 5-HTTLPR genotype and DRD2 genotype. Using Latent Growth Curve Modeling, OXTR genotype was not directly related to loneliness. An OXTR by sex interaction was found. Girls showed a steeper decline in loneliness when they had an A allele, compared to girls who were homozygous for the G allele. In addition, a gene-gene interaction or epistasis was observed. Both boys and girls who had at least one A1 allele for the DRD2 gene and also had the GG genotype for the OXTR gene showed stable levels of loneliness over time. The present study is the first to show that the GG genotype for the OXTR gene is linked with the development of loneliness in adolescence and that this association is moderated by participants’ sex and their genotype for a dopamine-related gene.

**Introduction**

Human beings are characterized by the need to belong (Baumeister & Leary, 1995), a fundamental need to bond with others. This need to belong motivates people to form and maintain interpersonal relationships. When the quantity or quality of these interpersonal relationships is not sufficient, people can experience loneliness (Baumeister & Leary, 1995). Loneliness is defined as the negative emotional response to a discrepancy between the desired and actual quality or quantity of one’s social relationships (Perlman & Peplau, 1981). Chronic feelings of loneliness can have serious consequences, such as major depressive disorder, personality disorders (Heinrich & Gullone, 2006), decreased cardiovascular health, and increased morbidity and mortality (Hawkley & Cacioppo, 2010). Given its detrimental consequences, it is important to examine antecedents of loneliness. Based on a recent theory of loneliness (Cacioppo & Hawkley, 2009) and emergent findings on the biological basis of social affiliation (e.g., Norman et al., 2011; Young & Wang, 2004), the present study aimed to expand current knowledge on the genetic basis of loneliness in adolescents. Specifically, we examined the role of the OXTR genotype, and its potential interactions with sex, parental support and 5-HTTLPR and DRD2 genotypes.

**Neurobiology and Genetics**

Recently, Cacioppo and Hawkley (2009) proposed a loneliness model in which lonely people are characterized by (a) hypervigilance for social threats and (b) diminished pleasure derived from positive (social) events and stimuli. These two characteristics lead to increasingly negative social expectations, which may sustain ongoing feelings of loneliness. This model can provide a theoretical basis for recent empirical findings that suggested genetic influences on loneliness in adolescence (Lucht et al., 2009; van Roekel et al., 2011; van Roekel, Scholte, et al., 2010).

The first characteristic, hypervigilance to social threats, may be related to the neuropeptide oxytocin. From administration studies, in which oxytocin is intranasally administered to participants, it appeared that higher oxytocin levels are related to decreased reactivity to threat-related faces or stimuli in males (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Petrovic, Kalisch, Singer, & Dolan, 2008). In a study on both males and females, higher levels of administered oxytocin were associated with decreased reactivity to threatening human stimuli (Cacioppo & Hawkley, 2009) and emergent findings on the biological basis of social affiliation (e.g., Norman et al., 2011; Young & Wang, 2004), the present study aimed to expand current knowledge on the genetic basis of loneliness in adolescents. Specifically, we examined the role of the OXTR genotype, and its potential interactions with sex, parental support and DRD2 and 5-HTTLPR genotypes.
The effects of oxytocin might be dependent on a polymorphism in the oxytocin receptor gene (OXTR rs53576), which encodes for two allelic variants, the A allele (i.e., the minor allele) and the G allele (i.e., the major allele). A previous study examining loneliness and the OXTR gene in a Caucasian sample has shown that adults carrying the AA genotype of the rs53576 variant of this gene had higher levels of loneliness than people carrying a G allele (Lucht et al., 2009). Studies examining other internalizing problems found contradictory results. Some studies also found the A allele to be related to less adaptive behaviour, such as less sensitive parenting (Caucasian sample, Bakermans-Kranenburg & van IJzendoorn, 2008), less empathy (mixed sample, Rodrigues et al., 2009), less sociality (Caucasian sample, Tost et al., 2010), higher levels of stress (mixed sample, Rodrigues et al., 2009), and lower levels of optimism, mastery and self-esteem (mixed sample, Saphire-Bernstein, Way, Kim, Sherman, & Taylor, 2011), whereas another study found the GG genotype to be related to higher levels of unipolar depression, and increased levels of separation anxiety and fearful attachment in patients with unipolar depression (sample ancestry not reported, Costa et al., 2009).

In addition to oxytocin, hypersensitivity to social threats is often associated with the serotonin system in the brain. People with the short variant (i.e., the minor allele) of the 5-HTTLPR gene showed hypersensitivity to threat-related signals (Heinz et al., 2005; Pezawas et al., 2005). A direct relation between this gene and loneliness was found in a study on adolescents, in which the short allele of the serotonin transporter gene (5-HTTLPR) was found to be related to the development of loneliness in adolescence (van Roekel, Scholte, et al., 2010). Adolescents carrying this allele remained relatively stable in their feelings of loneliness throughout adolescence, whereas adolescents with the long-long genotype showed a decrease in loneliness in adolescence.

The second characteristic, the diminished pleasure derived from social events, is also likely to be associated with oxytocin. Oxytocin receptors are highly expressed in reward areas of the brain (e.g., nucleus accumbens) and this neuropeptide can facilitate brain dopamine release (Young & Wang, 2004), which provides feelings of reward. Increased levels of oxytocin are related to greater reward dependence (Bora et al., 2009), indicating that less oxytocin may be related to experiencing less reward. This association may indicate that oxytocin plays a role in the lowered social reward experiences that accompany loneliness. As mentioned before, the dopamine system is also important in experiencing reward. The A1 allele (i.e., the minor allele) of the dopamine D2 receptor gene (DRD2) was found to be associated with reduced dopamine D2 receptor binding in the striatum, a brain area that is part of the reward system (Thompson et al., 1997), and individuals who carry at least one A1 allele are less sensitive to reward than are people with the A2A2 genotype (i.e., the major allele) (Blum et al., 1996). These results indicate that people with the A1 allele may be less susceptible to rewards in general, including social types of rewards. Research on the DRD2 gene in relation to loneliness revealed no direct effects of this genotype on adolescent loneliness (van Roekel et al., 2011).

Because the neuropeptide oxytocin plays a role in both characteristics that are found to be related to loneliness (Cacioppo & Hawkley, 2009), we will examine the relations between the OXTR genotype and adolescent loneliness in the present study. Because of the sex differences found in the effects of intranasally administered oxytocin, we will also examine the interaction between the OXTR genotype and sex. In addition, because the 5-HTTLPR and DRD2 genotype are related to mechanisms underlying loneliness, OXTR by 5-HTTLPR and OXTR by DRD2 interactions will be examined. We will not investigate main effects of 5-HTTLPR and DRD2 genotypes, because those effects have already been examined in the present sample of adolescents (van Roekel et al., 2011; van Roekel, Scholte, et al., 2010).

Gene – Environment Interactions With Parental Support

Previous studies on loneliness have indicated that parental support is an important environmental factor that is related to loneliness in adolescence (e.g., Mahon et al., 2006). A possible explanation for this finding is that a high quality of the relationship with parents is related to better social skills and relational competence, which in turn may lead to lower levels of loneliness (Engels, Finkenauer, Meeus, & Dekovic, 2001). In addition, two studies have found that perceived parental support interacts with the 5-HTTLPR genotype and the DRD2 genotype in predicting loneliness (van Roekel et al., 2011; van Roekel, Scholte, et al., 2010). More specifically, adolescents carrying a short allele of the 5-HTTLPR gene had lower loneliness scores when they experienced higher levels of maternal support. Adolescents with at least one A1 allele of the DRD2 gene were not affected by parental support, whereas adolescents with the A2A2 genotype had lower levels of loneliness when they experienced higher levels of paternal support. For the OXTR genotype, no gene-environment interactions have been examined in relation to loneliness. Because oxytocin receptors are highly prevalent in the reward areas of the brain (Young & Wang, 2004), one may expect that adolescents with certain OXTR gene variants are more susceptible to parental support, and therefore experience lower levels of loneliness.

The Present Study

The aim of the present study was to examine the relations between the OXTR genotype and adolescent loneliness, using a longitudinal 5-wave design. A previous study has examined the relation between the OXTR gene and loneliness (Lucht et al., 2009), but replication of these results is necessary. In addition, the previous study focused on adults, whereas we examined this relation in adolescents.

Little is known about the functional roles of the different variants of the OXTR gene, but results from the study on loneliness (Lucht et al., 2009) and other studies (Bakermans-Kranenburg & van IJzendoorn, 2008) imply that the A allele may be the ‘risk’ variant of the gene. Therefore, we hypothesized that adolescents with at least one A allele would have the highest levels of loneliness. Because it is not clear how the effects of the OXTR
gene may differ for boys and girls, we did not have a specific hypothesis for the OXTR by sex interaction. For the interactions with parental support, we expected adolescents who carried the A allele and experienced low levels of support to have the highest levels of loneliness, compared to adolescents carrying the GG genotype. In addition, we hypothesized that adolescents carrying both the A allele of the OXTR gene and the short allele of the 5-HTTLPR gene would have the highest levels of loneliness. For the interaction with the DRD2 genotype, adolescents carrying an A allele of the OXTR gene and at least one A1 allele were expected to have the highest levels of loneliness.

Method

Procedure
For the present study, data were used from the Family and Health study, a longitudinal Dutch survey study with five annual waves. This survey study aimed to examine different socialization processes in relation to various health behaviors in adolescents and their families (Harakeh et al., 2005). Through municipalities, families with two adolescent children were invited to participate. Of the families that responded, 885 families fulfilled the following criteria: parents were married or living together, all family members were biologically related to each other, and participating siblings were neither twins nor mentally or physically disabled. Of this group, 428 families were selected to obtain an equal distribution of sibling dyads (i.e., boy–boy, girl–girl, boy–girl), and an equal division of educational levels. For a detailed description of the procedure, see van der Zwaluw et al. (2010).

Participants
The participating families consisted of two biological parents and two adolescents. Data from the younger adolescent in each family were used for the present study, because those adolescents were entering adolescence at T1, making it possible to examine loneliness throughout adolescence. The final sample consisted of 302 adolescents. The mean age at T1 was 13.4 years (SD = 1.1); 53.6% were girls. One third (32.8%) of the adolescents attended lower education (i.e., preparatory secondary school for technical and vocational training), one third (36.1%) intermediate general education (i.e., preparatory secondary school for college), and one third (29.8%) attended the highest level of secondary school (i.e., preparatory secondary school for university). A small group of adolescents were not born in the Netherlands (1.2%) and of this group 0.2% were not born in a European country.

Attrition analyses were conducted to examine whether adolescents who gave their consent for genotyping (participants; n = 302) differed from the adolescents who did not (drop-outs; n = 126). T-tests showed no significant differences between participants and drop-outs (p > .05) in loneliness, parental support or age. For educational level and sex, Chi square statistics were calculated to examine differences between drop-outs and participants. No differences were found between the two groups, neither for sex ($\chi^2[428] = .70, p = .40$) nor for educational level ($\chi^2[422] = 9.84, p = .08$).

Measures
Loneliness. We measured loneliness with the 12-item peer-related loneliness subscale of the Louvain Loneliness Scale for Children and Adolescents (LLCA, Marcoen et al., 1987) at all five time points. This instrument has been used extensively in previous studies (e.g., Vanhalst, Klimstra, et al., 2012). Sample items were “I feel abandoned by my friends” and “I feel sad because I have no friends”. The items were answered on a four-point scale, ranging from (1) never to (4) always. Scores ranged from 12 to 48, with higher scores reflecting higher levels of loneliness. Cronbach’s alpha ranged between .90 and .93 at the different time points.

Perceived parental support. Participants filled out a 12-item version of the Relational Support Inventory (RSI, Scholte et al., 2001) at T1, tapping aspects of emotional and instrumental support. Participants completed the questionnaires for fathers and mothers separately. Example items are: “My mother/father supports me in the things I do”, and “My mother/father explains or shows how I can make or do something”. Each item was rated on a 5-point Likert scale, ranging from (1) very untrue to (5) very true. Alpha was .76 for maternal support and .80 for paternal support.

OXTR genotyping. DNA was isolated from saliva using the Oragene system (DNA Genotek Inc., Kanata, Ontario, Canada). The OXTR polymorphism (rs53576) was genotyped using Taqman analysis (Taqman Allelic Discrimination assay ID: C___3290335__10, reporter 1: VIC – A-allele, forward assay; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 5 µl containing 10 ng of genomic DNA: 2.5 µl of Taqman Mastermix (2x; Applied Biosystems) and 0.0625 µl of the Taqman assay (40x) and 1.4375 µl of MilliQ. Each amplification was performed by an initial denaturation at 95°C for 12 min, followed by 40 cycles of denaturation at 92°C for 15 seconds and annealing/extension at 60°C for 1 min. Genotyping was performed on a 7500 Fast Real-Time PCR System. Genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems). Genotyping was performed in a COX-accredited laboratory at the Department of Human Genetics of the Radboud University Nijmegen Medical Centre in Nijmegen. Generally, 5% blanks as well as duplicates between plates were taken along as quality controls during genotyping. Hardy–Weinberg equilibrium (HWE) proportions were estimated from parental genotype information using the Markov–Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3 ( Raymond & Rousset, 1995). No deviations from Hardy-Weinberg equilibrium (HWE) were detected (p = .94). To maximize the power of the analyses, OXTR genotype was dummy coded into 0 (AA/AG) and 1 (GG).
**OXTR Gene in Relation to Trait Loneliness**

**DRD2 Genotyping**
The DRD2 Taq1 A>C polymorphism was genotyped using Taqman assay (assay ID: Taqman assay/C____7486676_10, reporter 1: VIC-A allele, reverse assay; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 10 ul containing 10 ng of genomic DNA, 5 ul of Taqman Mastermix (2x; Applied Biosystems), 0.125 ul of the Taqman assay and 3.875 ul of H2O. Genotyping was performed on a 7500 Fast Real-Time PCR System and genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems). No deviations from HWE were detected (p = .96). DRD2 genotype was dummy-coded into 0 (A2A2) and 1 (A1A2 and A1A1).

**5-HTTLPRI Genotyping**
Genotyping of the 5-HTTLPRI polymorphism in the SLC6A4 (5-HTT, SERT) gene was performed by simple sequence length analysis. PCR was on 50 ng genomic DNA using 10 pmol of forward primer (5'-GGGTTGCCGCTTGATGCC-3') and 10 pmol reverse primer (5'-GGGGACTGGAGCTGGACAAACACG-3'), 0.25 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen, Breda, The Netherlands) in a PCR buffer containing 0.3 M Tris-HCl (pH 8.5), 75 mM ammoniumsulfate and 7.5 mM MgCl2. The cycling conditions for the polymerase chain reaction started with 5 min at 92°C, followed by 35 cycles of 1 min at 92°C, 1 min at the optimized annealing temperature (57.5°C), and 1 min 72°C, then followed by an extra 5 min 72°C. PCR products were analyzed on a 2% agarose gel. The amplification yielded distinct bands at 484 bp (short “s” allele) and 528 bp (long “l” allele). No deviations from HWE were detected (p = .89). We dummy-coded the 5-HTTLPRI genotype into 0 (short-short and short-long) and 1 (long-long) to maximize the power of the analyses.

**Statistical Analyses**
Latent Growth Curve Modeling (LGCM) was used to estimate both the individual level of loneliness at baseline (i.e., intercept), and the change in loneliness over time (i.e., slope, Duncan et al., 2006). In this approach, it is assumed that all participants may start at a different level of loneliness at baseline and have different rates of change in loneliness over time: Individual growth is examined for each participant. Therefore, LGCM is an excellent way to examine individual variation in the development of loneliness and to investigate whether certain predictors are related to these changes over time. Mplus (Muthén & Muthén, 1998-2007), a statistical software program designed for structural equation modeling (SEM) analyses, was used for these analyses.

Parameters in the models were estimated by applying a method that corrects for the non-normal distribution of the dependent variables (i.e., the maximum likelihood estimator with robust standard errors, MLR). To deal with missing data, which were rare, we did not impute these data but borrowed information from the observed portion of the data. This approach (which is referred to as the full-information maximum likelihood or FIML approach) is superior to other techniques for handling missing data, such as pairwise deletion, or listwise deletion (Muthén & Muthén, 1998-2007).

First, the initial developmental model without predictors was tested. Second, the relation between the OXTR genotype and the baseline level (i.e., intercept) and rate of change (i.e., slope) in loneliness was examined. In addition, we tested whether this relation differed for boys and girls. Third, the main effect of maternal support and the interaction between maternal support and the OXTR genotype were examined. Fourth, and finally, gene-gene interactions (i.e., 5-HTTLPRI genotype X OXTR genotype and DRD2 genotype X OXTR genotype) were examined. We controlled for sex in all analyses. All variables were centered before computing the interaction terms, to avoid multi-collinearity. Model fit was assessed by the following global fit indices: χ², CFI (with a cut-off value of .95) and RMSEA (with a cut-off value of .06) (Hu & Bentler, 1999).

**Results**

**Descriptive Statistics**
The distributions of the different genotypes are presented in Table 1. Means, standard deviations, and Pearson correlations among model variables are depicted in Table 2.

The average level of loneliness across the five time points was relatively low in absolute terms, with means ranging from 17.67 to 18.81 (out of a maximum score of 48). These means were comparable to those observed in other studies on community samples (e.g., Marcoen & Goossens, 1993). Sex was dummy-coded (0 = boys; 1 = girls) so that the average score for sex in Table 2 represents the proportion of girls in the sample. The correlations show that the OXTR genotype is related to loneliness at T3 and T4, indicating that adolescents with the GG genotype show higher levels of loneliness at T3 and T4. Maternal support is related to lower levels of loneliness at T1, T3, T4, and T5, and paternal support to lower levels of loneliness at T1, T2, and T3.

**Table 1** Distribution of Genotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXTR genotype</td>
<td>AA = 28 (9.3%)</td>
<td>AG = 127 (42.1%)</td>
<td>GG = 147 (48.7%)</td>
</tr>
<tr>
<td>5-HTTLPRI genotype</td>
<td>SS = 55 (18.3%)</td>
<td>SL = 146 (48.5%)</td>
<td>LL = 100 (33.2%)</td>
</tr>
<tr>
<td>DRD2 genotype</td>
<td>A1A1 = 8 (2.6%)</td>
<td>A1A2 = 93 (30.8%)</td>
<td>A2A2 = 201 (66.6%)</td>
</tr>
</tbody>
</table>

Note: OXTR = Oxytocin receptor gene; 5-HTTLPRI = Serotonin transporter gene; DRD2 = Dopamine D2 receptor gene.

**Model Findings**
First, the initial developmental model (i.e., no predictors) was tested (Table 3). The intercept and slope were significant, indicating that the mean loneliness score at baseline was 18.82, and that the level of loneliness in general significantly decreased over time.
Second, we examined the main effect of OXTR genotype on the intercept and slope, while controlling for sex. In all models, we initially controlled for depressive symptoms (measured with a six-item questionnaire, Kandel & Davies, 1982), 5-HTTLPR and DRD2 genotypes, but this did not affect the results. Therefore, we decided not to control for these variables in the final analyses. The results for this model were not significant, that is, the OXTR genotype was not related to intercept nor slope. To examine whether the relation between OXTR genotype and the intercept and slope differed between boys and girls, we entered the interaction between sex and OXTR genotype in the model. No relation was found between the interaction and the intercept of loneliness. However, the interaction was significantly related to the slope of loneliness. This interaction is presented in Figure 1. In this figure, the Y axis represents the slope. Negative values for the slope of loneliness correspond with a steeper decrease in loneliness, whereas values close to zero correspond with stable levels of loneliness over the five waves. For boys, no difference was found between the slopes for A allele carriers and the slopes for adolescents homozygous for the G allele. However, girls had significantly steeper slopes when they carried an A allele, compared to girls with the GG genotype. As can be seen in Figure 1, girls with the GG genotype did not decrease in loneliness, as their slope was close to zero.

Third, maternal support was entered into the model. A significant negative relation was found with the intercept, indicating that adolescents who experienced high levels of

### Table 3 Regression of Initial Level (Intercept) and Rate of Change (Slope) in Adolescents’ Loneliness on Gene x Environment Interactions

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Intercept</th>
<th>Slope</th>
<th>$\chi^2$ (df)</th>
<th>CFI</th>
<th>RMSEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initial developmental model</td>
<td>18.82 (.35)**</td>
<td>-27 (.10)**</td>
<td>40.75 (10)</td>
<td>.91</td>
<td>.101</td>
</tr>
<tr>
<td>2. OXTR</td>
<td>-0.06 (.07)</td>
<td>.12 (.08)</td>
<td>52.38 (16)</td>
<td>.92</td>
<td>.087</td>
</tr>
<tr>
<td>2b. OXTR x Sex</td>
<td>-0.04 (.07)</td>
<td>.18 (.08)*</td>
<td>64.93 (19)</td>
<td>.90</td>
<td>.089</td>
</tr>
<tr>
<td>3. Support (mother)</td>
<td>-1.19 (.07)**</td>
<td>-0.03 (.10)</td>
<td>60.67 (19)</td>
<td>.91</td>
<td>.085</td>
</tr>
<tr>
<td>3b. OXTR x Support (mother)</td>
<td>.06 (.14)</td>
<td>-25 (.20)</td>
<td>65.67 (22)</td>
<td>.91</td>
<td>.081</td>
</tr>
<tr>
<td>4. Support (father)</td>
<td>-2.26 (.07)**</td>
<td>.17 (.10)</td>
<td>54.50 (19)</td>
<td>.92</td>
<td>.079</td>
</tr>
<tr>
<td>4b. OXTR x Support (father)</td>
<td>-1.17 (.14)</td>
<td>-0.04 (.19)</td>
<td>61.07 (22)</td>
<td>.92</td>
<td>.077</td>
</tr>
<tr>
<td>5. OXTR x 5-HTTLPR</td>
<td>-0.01 (.07)</td>
<td>-0.03 (.09)</td>
<td>60.31 (22)</td>
<td>.92</td>
<td>.076</td>
</tr>
<tr>
<td>6. OXTR x DRD2</td>
<td>-0.08 (.07)</td>
<td>.18 (.09)*</td>
<td>61.72 (22)</td>
<td>.92</td>
<td>.077</td>
</tr>
</tbody>
</table>

Note: OXTR = Oxytocin receptor gene. 5-HTTLPR = Serotonin transporter gene. DRD2 = Dopamine D2 receptor gene. AA = A/A; AG = A/G; GG = G/G. In all analyses, we controlled for sex. Only new variables entered in the model are depicted in the table.
Discussion

The present study examined the relation between the OXTR genotype and loneliness in adolescence, including gene-environment interactions with parental support and gene-gene interactions with DRD2 and 5-HTTLPR genotypes. One of the main findings was that girls carrying the GG (OXTR) genotype remained stable in their feelings of loneliness over time, whereas the normative trend is to decrease in loneliness throughout adolescence. In addition, a significant interaction was found between the OXTR and the DRD2 genotype, in which adolescents carrying both the GG genotype (OXTR) and at least one A1 allele (DRD2) remained stable in loneliness over time, whereas adolescents with other combinations of genotypes decreased in loneliness.

Genes, Environments, and Interactions

In the present study, no direct relation was found between the OXTR genotype and loneliness in the total sample. However, our results indicate that the OXTR gene may have different effects in boys and girls. For boys, the OXTR genotype was neither related to their baseline levels of loneliness, nor the development of loneliness over time. However, girls with the GG genotype remained stable in loneliness over time. A possible explanation why boys’ loneliness was not affected by the OXTR gene may be that oxytocin receptors...
are partly upregulated by estrogen (e.g., Bale & Dorsa, 1995; M. Feng et al., 2009; Quiriones-Jenab et al., 1995), a sex hormone which is particularly present in females. Therefore, the effects of the OXTR gene may be more pronounced in adolescent girls. Our results indicate that the G allele of the OXTR gene may be the ‘risk’ variant of the gene and that this genotype affects the development of loneliness in girls.

This result is in contrast with previous studies on loneliness (Lucht et al., 2009) and on other outcome variables such as sensitive parenting and empathy (e.g., Bakermans-Kranenburg & van IJzendoorn, 2008; Rodrigues et al., 2009), in which the A allele was found to be the ‘risk’ variant of the OXTR gene. Nonetheless, Costa et al. (2009) found that unipolar depression was more prevalent in people with GG genotypes, and in those patients with unipolar depression, the GG genotype was related to higher levels of separation anxiety and fearful attachment, which is in line with our finding indicating that the GG genotype is the ‘risk’ variant. These contradictory results may be explained by age effects: our sample consisted of adolescents, whereas other samples exclusively included adults. Previous studies on other genes have shown that effects of genes can be age-dependent, and therefore the ‘risk’ allele can differ between adolescence and adulthood (e.g., Hilt, Sander, Nolen-Hoeksema, & Simen, 2007).

Interestingly, we found significant correlations between the OXTR genotype and loneliness at T3 and T4. However, we do not have an explanation why OXTR is related to loneliness at these specific time points. To our knowledge, there are no specific developments at that time points that could explain these correlations. Further research is necessary to further examine these correlations.

Our results provided no evidence for gene-environment interactions. This ran counter to our expectations and previous research on loneliness, in which interactions were found between perceived parental support and the 5-HTTLPR and DRD2 genotypes (van Roekel et al., 2011; van Roekel, Schofte, et al., 2010). A possible explanation for this negative finding may be that the current gene-environment interaction is particularly present in girls, because the effects of the OXTR genotype differed for boys and girls. However, we were not able to examine these sex differences as this requires a larger sample size. In addition, we were the first to examine a gene-environment interaction with the rs53576 polymorphism of the OXTR gene. It may be that this particular gene does not interact with the environment as we expected. Future studies may examine whether concepts that are more directly related to oxytocin functioning interact with the OXTR genotype. For example, communication with parents may be more directly affected by oxytocin, because oxytocin is specifically involved in social interactions and social approach behavior (IsHak, Kahloon, & Fakhry, 2010).

Based on a recent loneliness model (Cacioppo & Hawkley, 2009), we hypothesized that the OXTR genotype would interact with both the DRD2 and 5-HTTLPR genotypes. We hypothesized that adolescents with the A allele (OXTR) and an A1 allele (DRD2) would be more at risk for loneliness, which was not confirmed by the results. Results showed a significant interaction between the OXTR and the DRD2 genotype, in which adolescents carrying both the GG genotype (OXTR) and at least one A1 allele (DRD2) remained stable in loneliness over time, whereas adolescents with other combinations of genotypes decreased in loneliness. As mentioned before, our results imply that the GG genotype may be the ‘risk’ variant of the gene. In that way, it could be that people with the GG genotype are less sensitive to rewards, because decreased levels of oxytocin may be related to experiencing less reward (Bora et al., 2009).

As people with the A1 allele are also less sensitive to rewards (Blum et al., 1996), it may be that adolescents who carry both the A1 allele (DRD2) and the GG genotype (OXTR) are less sensitive to rewards in general, including social types of rewards. Because these adolescents might feel less rewarded by social stimuli, they will not actively strive to interact with others and therefore, their feelings of loneliness throughout adolescence will not decrease. On the other hand, adolescents with other combinations of genotypes, who are rewarded by social stimuli, could actively seek out social interactions and hence decrease in their feelings of loneliness over time. Our results could indicate that carrying this particular combination of alleles implies a risk for developing loneliness.

No interaction was found between the OXTR genotype and the 5-HTTLPR genotype, which was in contrast with our expectations6. We expected adolescents with the short allele of the 5-HTTLPR gene and the A allele of the OXTR gene to have higher levels of loneliness compared to adolescents with other combinations of genotypes. A previous study on sensitive parenting did find an interaction between 5-HTTLPR and OXTR in adult females (Bakermans-Kranenburg & van IJzendoorn, 2008). As mentioned before, gene effects can be age-dependent (e.g., Hilt et al., 2007), which could explain differences in findings between adolescent and adult samples. In addition, the adult sample may have been an at-risk sample, because the participants were selected to participate when their children exhibited clinical levels of externalizing problems (Bakermans-Kranenburg & van IJzendoorn, 2008). The present group of adolescents was a normative population sample, which may also explain the differences in findings.

Limitations and Suggestions for Future Research

One of the strengths of the present study is the longitudinal design, making it possible to examine gene effects on the development of loneliness throughout adolescence. However, the study also has some limitations that should be acknowledged. First, our sample size was not large enough to examine whether the gene-environment interactions and gene-gene interactions differed for boys and girls. Because our results

---

6 Importantly, the lack of gene-gene interaction between OXTR and 5-HTTLPR was not likely to be due to sample size issues, as the genotype distribution of the different genotype combinations did not result in very small subgroups (e.g., the smallest group consisted of 50 adolescents). In addition, the sample sizes for the different genotype combinations were comparable to those for DRD2 and OXTR, for which we did find a significant gene-gene interaction.
showed a significant OXTR by sex interaction, it would be interesting to examine whether the gene-environment interactions and gene-gene interactions are particularly present for girls. Due to our relatively small sample size for the specific hypotheses tested, our study may have been underpowered. However, we examined whether we had enough power to find gene-environment interactions. We conducted power analyses using the software Quanto (Gauderman & Morrison, 2006). For the power calculation we applied the gene-environment design option for continuous outcomes with independent individuals. Further, it was assumed that approximately 30% of the sample would have the OXTR-A allele (Lucht et al., 2009). The assumed inheritance model was dominant. Finally, the assumed main effects for the OXTR genotype and parental support were 0.01 and 0.05, respectively. To detect a small effect for the gene-environment interaction with an $R^2$ of 0.02 to 0.03, with 80% power (alpha = .05), the sample size required is between 242 and 365. With our sample size of 302 adolescents, we should have been able to detect a small effect size of the OXTR x support interaction. Still, future studies should include larger samples to examine these relations in further detail. In addition, replication of our results is necessary, in community samples as well as at-risk or clinical samples. Second, we were only able to examine gene-environment interactions with parental support, whereas other sources of support, such as peers, may also be important (e.g., Asher & Paquette, 2003; Benjet, Thompson, & Gotlib, 2010). In addition, it may be that more negative environmental variables such as negative life events or childhood abuse (Uher & McGuffin, 2008) would interact with the OXTR gene, which could be examined in future studies. However, it should be mentioned that significant gene x support interactions have been found in previous studies on loneliness (van Roekel et al., 2011; van Roekel, Scholte, et al., 2010). Third, previous studies have shown that other SNPs in the OXTR gene may also be important (e.g., rs2254298; (Costa et al., 2009) and rs2268498 (Montag, Fiebach, Kirsch, & Reuter, 2011)), whereas we exclusively had data on the rs53576 SNP. Future studies should consider examining those SNPs in relation to loneliness as well. Fourth, research by Neville et al. (2004) has shown that the DRD2 Taq1A SNP is not located in the DRD2 gene, but in a neighboring gene, the ankyrin repeat and kinase domain containing 1 (ANKK1). Studies on alcohol dependence (Dick et al., 2007) and nicotine dependence (Gelernter et al., 2006) have indeed suggested that the relation between DRD2 and alcohol and nicotine dependence may be attributable to variants of the ANKK1 gene. Future research should therefore examine relations between the ANKK1 gene and loneliness. Fifth, population stratification may affect study findings (Marchini et al., 2004). However, in our study there was only a small group of adolescents who were not born in the Netherlands (1.2%), and of this group only 0.2% were not born in a European country. In addition, the genotype frequencies of OXTR, DRD2, and 5-HTTLPR were all in line with those of Caucasian samples in other studies. Therefore, we may conclude that it is not likely that population stratification has affected our results.

Conclusions

In sum, results from the present study imply that the rs33576 polymorphism of the OXTR gene plays a role in the development of loneliness in girls. Specifically, girls with the GG genotype remain stable in their feelings of loneliness throughout adolescence, whereas a decrease is normative in adolescence. In addition, adolescents who carry both the GG genotype (OXTR) and an A1 allele (DRD2) also remain stable in their feelings of loneliness. No interactions were found between OXTR and perceived parental support. Because this study is the first to examine the relation between the OXTR gene and loneliness and the first to examine OXTR by environment and OXTR by gene interactions, it is important to stress that replication of these findings is pivotal.
Chapter 10
The oxytocin receptor gene (OXTR) in relation to state levels of loneliness in adolescence: Evidence for micro-level gene-environment interactions

Published as:
CHAPTER 10

OXTR GENE IN RELATION TO STATE LONELINESS

Introduction

The need to belong hypothesis states that every human being has an innate drive to form and maintain a certain number of relationships with other humans (Baumeister & Leary, 1995). When this need is not fulfilled, people can experience social pain, for example in the form of feelings of loneliness. From an evolutionary perspective, feelings of loneliness can be functional and adaptive (Cacioppo, Hawkley, et al., 2006). When a person experiences loneliness in response to social isolation, that person might be more likely to be activated to go out and initiate or restore social relationships than people who do not experience loneliness in response to social isolation. In turn, people who do experience loneliness are more likely to survive and pass on their genes, because the likelihood of survival is greater in a social community in which food is shared and people are protected from outside threats through stable social relationships. From this point of view, experiencing levels of loneliness that are transient (i.e., state loneliness) is not necessarily negative, and may even have positive consequences as a person experiencing state loneliness may be motivated to actively seek social contact. In contrast, levels of loneliness that are chronic and enduring (i.e., chronic levels of trait loneliness) are found to have negative consequences, such as cardiovascular disease, sleep problems, and depression (e.g., Heinrich & Gullone, 2006).

From an evolutionary point of view, it can be expected that state levels of loneliness have a genetic basis, as these feelings may be adaptive for survival. Yet genetic studies have exclusively examined trait levels of loneliness. Behavioral genetic studies have found that trait levels of loneliness are moderately heritable, with heritability estimates ranging from 45 to 55% in children (Bartels et al., 2008; McGuire & Clifford, 2000), 75% in adolescents (Waaktaar & Torgersen, 2012), and 48% in adults (Boomsma et al., 2005). Further molecular-genetic research has shown that several genes, amongst which the oxytocin receptor gene (OXTR), are related to trait levels of loneliness in adolescents (Lucht et al., 2009; van Roekel, Verhagen, Engels, Goossens, & Scholte, 2013). As no studies have examined the genetic basis of state levels of loneliness, the goal of the present study was to examine relations between a variant in the OXTR gene (rs53576) and state levels of loneliness in early adolescents.

Abstract

Previous research has shown that the rs53576 variant of the oxytocin receptor gene (OXTR) is associated with trait levels of loneliness, but results are inconsistent. The aim of the present study is to examine micro-level effects of the OXTR rs53576 variant on state levels of loneliness in early adolescents. In addition, gene-environment interactions are examined between this OXTR variant and positive and negative perceptions of company. Data were collected in 278 adolescents (58% girls), by means of the Experience Sampling Method (ESM). Sampling periods consisted of six days with nine assessments per day. A relation was found between the OXTR rs53576 variant and state loneliness, in girls only. Girls carrying an A allele had higher levels of state loneliness than girls carrying the GG genotype. In addition, adolescents with an A allele were more affected by negative perceptions of company than GG carriers, on weekend days only. No significant gene-environment interactions were found with positive company. Adolescents carrying an A allele were more susceptible to negative environments during weekend days than GG carriers. Our findings emphasize the importance of operationalizing the phenotype and the environment accurately.
related to less sensitive parenting (Bakermans-Kranenburg & van IJzendoorn, 2008), less empathy and higher levels of stress (Rodrigues et al., 2009), less sociality (Tost et al., 2010), and less optimism and self-esteem (Saphire-Bernstein et al., 2011), although the relation between OXTR and optimism was not replicated in a different sample (Cornelis et al., 2012). In addition, A carriers displayed less nonverbal affiliative cues in social interaction and were rated as less prosocial than GG carriers (Kogan et al., 2011). In contrast, another study found that patients with unipolar depression who carried the GG genotype had higher levels of separation anxiety and fearful attachment, compared to patients carrying an A allele (Costa et al., 2009).

OXTR Gene and Loneliness

Regarding trait levels of loneliness, two studies have examined relations with the OXTR rs53576 genetic variant. A study on adults found that AA carriers experienced slightly higher levels of loneliness than people carrying a G allele (Lucht et al., 2009), which was only present for males, not females. Further, a study on adolescents found a sex difference throughout adolescence, in that girls carrying an A allele showed a steeper decline in loneliness, compared to girls carrying the GG genotype (van Roekel et al., 2013). These findings indicate that the AA genotype conveys a risk for loneliness in male adults (Lucht et al., 2009), whereas the GG genotype can be considered a risk for relatively stable levels of loneliness throughout adolescence for girls (van Roekel et al., 2013).

As main effects of genes in mental disorders are often small and difficult to detect, it is important to operationalize the phenotype accurately and precisely. A solution for this problem may lie in examining micro-level effects of the OXTR gene. Therefore, we will examine state levels of loneliness in daily life, by using the Experience Sampling Method (Myin-Germeys et al., 2009). In this way, adolescents report on their actual feelings of loneliness in real life. The main advantages of this method are that (a) it prevents recall bias because participants report on what they are experiencing at that moment and (b) the ecological validity is high, as participants fill out the questionnaires in their natural environment.

Gene-Environment Interactions

In a recent review, Bartz et al. (2011) argue that oxytocin affects the perception of and attention to social cues. This could indicate that individuals with higher oxytocin levels are more sensitive to social cues, and hence also more affected by those social cues than individuals with lower oxytocin levels. These findings concur with the Differential Susceptibility Theory, which states that some individuals are more susceptible to their environment due to neurobiological factors (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011). Hence, in the present study we will examine gene-environment interactions as well.

The only study examining GxE interactions with the rs53576 variant of the OXTR gene in relation to trait loneliness (van Roekel et al., 2013) did not find any interactions. Regarding other outcomes findings are mixed. Two studies found that GG genotypes were more affected by their environment, in that people carrying the GG genotype experienced more negative outcomes (i.e., lower maternal sensitivity (Sturge-Apple et al., 2012), higher emotion dysregulation and disorganized attachment (Bradley et al., 2011)) when they experienced more negative environments (i.e., high levels of interparental conflict or maltreatment), and experienced more positive outcomes when they experienced more positive environments (low levels of interparental conflict or maltreatment). These studies indicate that GG genotypes may be more negatively affected by negative environments than A carriers. In addition, another study found that G carriers (i.e., GG and AG genotypes) showed lower stress responses after receiving social support, compared to AA genotypes who did not benefit from social support (Chen, Kumsta, et al., 2011). In contrast, another study examining physical health problems found that for A carriers, exposure to more stressful life events was related to new-onset ailments, whereas there was no relation between stressful life events and ailments in GG genotypes (Poulin & Holman, 2013). These findings indicate that A carriers would be more susceptible to negative environments than GG genotypes.

In the present study, we will examine state-level gene-environment interactions. Previous research on state levels of positive and negative affect has shown that the perceptions adolescents have of their company (i.e., positive or negative) are related to their levels of positive and negative affect (van Roekel et al., 2013). Therefore, we expect that adolescents’ perceptions of their company will be related to state levels of loneliness. Hence, we will examine whether positive and negative perceptions of company are related to state levels of loneliness, and whether these relations are moderated by the OXTR genotype.

Sex Differences

Oxytocin receptors are partly upregulated by estrogen (e.g., Feng et al., 2009), a sex hormone which is particularly present in females. This can explain why some studies reported sex differences in the effects of the OXTR gene. For example, Tost and colleagues (2010) found that the relation between OXTR and the brain volume of the hypothalamus and amygdala differed between males and females. Lucht et al. (2009) showed that the relation between OXTR and positive affect was only present for males, but not for females. In Kogan et al. (2011), marginally significant differences in prosociality were found between male and female GG genotypes. Importantly, some other studies did not find any sex differences (Rodrigues et al., 2009; Saphire-Bernstein et al., 2011), or examined females only (Bakermans-Kranenburg & van IJzendoorn, 2008; Sturge-Apple et al., 2012). Regarding trait levels of loneliness, it was found that the relation between the OXTR gene and the development of loneliness in adolescence was only present for girls (van Roekel et al., 2013). Because of these sex-dependent findings, it is important to consider sex differences in the effects of the OXTR gene.
The Present Study

The main aim of this study was to examine relations between the OXTR rs53576 genotype and state levels of loneliness. As previous findings on the associations between this OXTR genotype and trait levels of loneliness were inconsistent, we did not have specific hypotheses regarding direct relations between OXTR and state levels of loneliness. Second, we examined gene-environment interactions between perceptions of company and OXTR on state levels of loneliness. As previous studies have shown that GG genotypes were more negatively affected by negative environmental factors (Bradley et al., 2011; Sturge-Apple et al., 2012), we hypothesized that the relation between negative perceptions of company would be stronger for adolescents carrying the GG genotype. As no studies have examined GxE interactions with positive environmental factors (e.g., the GxE studies described earlier only examined the absence of negative environments such as conflict or maltreatment), we did not have a specific hypothesis for the interaction between OXTR and positive perceptions of company.

Importantly, because adolescents are obliged to go to school during weekdays, the range of people whom they can choose to spend their time with is limited during weekdays. In weekends, on the other hand, adolescents can choose their company. This difference between week and weekend days may affect how adolescents perceive their company and the relations between these perceptions of company and state loneliness. Therefore, we also examined whether perceptions of company and state levels of loneliness differed between week and weekend days, and whether the interaction between OXTR and perceptions of company had different effects on state loneliness during week or weekend days. Finally, as mentioned before, because sex differences are present in the effects of the OXTR gene, all analyses were tested for boys and girls separately.

Method

Ethics Statement

The present study was approved by the Committee on Research Involving Human Subjects (CMO Arnhem-Nijmegen, 2009, No. 285). Both adolescents and their parents had to sign a consent form in order to participate in the study.

Participants

The total sample consisted of 301 adolescents (39% boys) from four secondary schools. The age of the participants ranged between 13 and 16 years (M = 14.19; SD = .55). The majority of the adolescents (97.1%) were born in The Netherlands and only 1.3% of the adolescents was not born in an European country. Educational levels were equally distributed (i.e., 24% preparatory secondary school for technical and vocational training, 36% preparatory school for college, 40% preparatory school for university).

Procedure

Adolescents were recruited through high schools. Schools were sent information letters in which they were asked to participate in the present study. In schools that provided their consent, all second-year students and their parents received a letter in which they were asked to participate.

The study consisted of (a) a baseline questionnaire, (b) saliva collection for genetic analyses, and (c) the Experience Sampling Method (ESM) period. For a detailed description of the procedure, see (van Roekel et al., 2013). The baseline questionnaire was administered online during school hours, after which they were asked to provide saliva (Oragene, DNA Genotek Inc., Ottawa, ON, Canada). The ESM period took place three to eight weeks after the baseline questionnaire and always started on Fridays. Adolescents received a smartphone, on which a program was installed (http://myexperience.sourceforge.net) that emitted buzzing signals at nine random time points each day, for six consecutive days. When adolescents received a signal, they had to immediately pause their activity and fill out the questionnaire on the smartphone. Data were stored on the smartphones and a text message was sent to the principal investigator after each completed questionnaire, making it possible to check compliance. Adolescents received the full reward of € 20 (i.e., about 27 US $) when they completed at least 55% of the momentary assessments.

Materials

State loneliness. We used four items to measure state levels of loneliness: lonely, isolated, left out, and abandoned. Adolescents had to rate at each momentary assessment to what extent they experienced the described emotion on a 7-point scale, ranging from (1) not at all to (7) very much. Cronbach’s alpha was calculated for each momentary assessment separately, and then averaged over all momentary assessments, which resulted in an alpha of .73. Inter-item correlations ranged from r = .43 to r = .90.

Perceptions of company. When adolescents reported that they were with other people at the time of the ESM signal, positive and negative perceptions of company were measured (from now on referred to as ‘positive company’ and ‘negative company’). Positive company consisted of the items “I feel accepted by this company” and “I feel comfortable in this company” (r = .60). Negative company consisted of the items “I feel threatened by this company” and “I feel judged by this company” (r = .37).

OXTR genotyping. DNA was isolated from saliva using the Oragene system (DNA Genotek Inc., Kanata, Ontario, Canada). The OXTR polymorphism rs53576 was genotyped using Taqman analysis (Taqman Allelic Discrimination assay ID: C___3290335_10, reporter 1: VIC-A-allele, forward assay; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 5 µl containing 10 ng of genomic DNA, 2.5 µl...
of Taqman Mastermix (2x; Applied Biosystems) and 0.0625 µl of the Taqman assay (40x) and 1.42575 µl of MasterMix. Each amplification was performed by an initial denaturation at 95°C for 12 min, followed by 40 cycles of denaturation at 92°C for 15 seconds and annealing/extension at 60°C for 1 min. Genotyping was performed on a 7500 Fast Real-Time PCR System. Genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems). Genotyping was performed in a laboratory at the Department of Human Genetics of the Radboud University Nijmegen Medical Centre in Nijmegen, which is accredited by the leading institute for laboratories in the health care sector in the Netherlands (called CCKL). Generally, 5% blanks as well as duplicates between plates were taken along as quality controls during genotyping. No deviations from Hardy-Weinberg equilibrium (HWE) were detected ($p = .87$). To maximize the power of the analyses, the OXTR genotype was dummy coded into 0 (GG) and 1 (AA/AG).

**Power Analysis**

We conducted power-analyses using the software Quanto to test whether we had enough power for the gene-environment interactions (Gauderman & Morrison, 2006). For the power calculation we applied the gene-environment design option for continuous outcomes with independent individuals. Further, it was assumed that approximately 30% of the sample would have the OXTR-A allele (Lucht et al., 2009). The assumed inheritance model was dominant. Finally, the assumed main effects of the OXTR genotype and negative company were 0.02 and 0.18, respectively. To detect a small effect for the gene by sex interaction with an $R^2$ of 0.02 to 0.03, with 80% power ($alpha = .05$), the sample size required is between 205 and 310. For positive company (assumed main effect was 0.13), we needed a sample size between 218 and 330 to detect a small effect of the gene-environment interaction with an $R^2$ of 0.02 to 0.03 and 80% power ($alpha = .05$). This indicates that with our sample size of 275 adolescents, we had enough power to detect a small effect size of the OXTR x company interactions.

**Statistical Analyses**

We examined relations between the OXTR genotype and state levels of loneliness. Because our repeated momentary assessments (Level 1) were nested within individuals (Level 2), we conducted multilevel linear regression analyses in Mplus (Muthén & Muthén, 1998-2007). To examine possible sex differences in the relations between the OXTR genotype and state levels of loneliness, we conducted multi-group analyses across sex. We did this by examining whether the model fit for the model in which the paths were allowed to differ between boys and girls was significantly better than the model fit for the model in which the paths were constrained to be equal for boys and girls, using a chi-square difference test ($Delta \chi^2$; Kleinjan et al., 2009). If significant differences between boys and girls would emerge, we further compared differences between boys and girls per path, by examining whether the model fit of the model in which the path of interest was allowed to differ between boys and girls was better than the model fit for the model in which all paths were constrained, also by using the chi-square difference test.

Level 1 predictors (i.e., positive and negative company) were centered at group-level and were included in the model as random coefficients. In this way, it is possible to examine whether the relation between the Level 1 variables vary across adolescents (Hox, 2010). Hence, by using this approach, we can examine whether the relation between the Level 1 variables (positive and negative company) and state loneliness varies across adolescents, by including positive and negative company as random predictors in the model. When these coefficients are significant, this implies that the relations between negative company and affect differ between adolescents, and can therefore be predicted by individual characteristics, such as the OXTR genotype.

First, we tested the empty model without predictors. Second, the OXTR genotype was added to the model to examine relations between the genotype and state loneliness. Third, we examined relations between positive and negative company and state loneliness, in two separate models. Fourth, the interactions between the OXTR genotype and negative and positive company were examined over all sampling days. Next, we split the analyses for week days and weekend days to further examine whether the interaction between OXTR and negative and positive company had different effects on state loneliness during week or weekend days.

Finally, as the inter-item correlation between the two negative company items was relatively low, we checked in additional analyses whether the results involving negative company differed between these two items (i.e., I feel judged by this company, I feel threatened by this company).

**Results**

**Descriptive Statistics**

Means and standard deviations for the model variables are depicted in Table 1, separately for boys and girls. Mean levels of state loneliness were relatively low, compared to the range (i.e., 1-7). In Figure 1, state levels of loneliness are depicted across the ESM period, split for boys and girls. As can be seen in this Figure, state levels of loneliness were lowest during weekend days ($t = 4.49, p < .001$), for both boys and girls.

For the OXTR genotype, 110 adolescents (41.9%) carried the GG genotype (43 boys and 67 girls), 122 adolescents (46.6%) carried the heterogenous genotype (51 boys and 71 girls), and 30 adolescents (11.5%) were homozygous for the A allele (12 boys and 18 girls). No sex differences were found for any of the variables.

Next, correlations between model variables were examined (see Table 2). For boys, no significant correlations were found between the OXTR genotype and the model variables. In contrast, for girls we found small positive correlations between the OXTR
CHAPTER 10

OXTR GENE IN RELATION TO STATE LONELINESS

Table 1 Descriptive Statistics, Split for Boys and Girls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boys</th>
<th>Girls</th>
<th>t</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>State loneliness*</td>
<td>1.30 (0.40)</td>
<td>1.36 (0.43)</td>
<td>-1.21</td>
<td>274</td>
</tr>
<tr>
<td>Positive company*</td>
<td>6.14 (0.65)</td>
<td>6.15 (0.57)</td>
<td>-0.10</td>
<td>274</td>
</tr>
<tr>
<td>Negative company*</td>
<td>1.52 (0.58)</td>
<td>1.56 (0.51)</td>
<td>-0.62</td>
<td>274</td>
</tr>
</tbody>
</table>

Note: *For the momentary assessment data, aggregated mean scores were calculated within persons.

Figure 1 Levels of state loneliness during the ESM period, split for boys and girls.

Table 2 Correlations Between Model Variables, Split for Boys and Girls

<table>
<thead>
<tr>
<th>Variable</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. OXTR genotype</td>
<td></td>
<td>.20*</td>
<td>.06</td>
<td>.19*</td>
</tr>
<tr>
<td>2. State loneliness</td>
<td>-.14</td>
<td></td>
<td>-.51**</td>
<td></td>
</tr>
<tr>
<td>3. Positive company</td>
<td>.07</td>
<td>-.67**</td>
<td></td>
<td>-.65**</td>
</tr>
<tr>
<td>4. Negative company</td>
<td>-.10</td>
<td>.70**</td>
<td>-.72**</td>
<td></td>
</tr>
</tbody>
</table>

Note: *p < .05; **p < .001. *0 = GG, 1 = AA/AG
Above the diagonal correlations for girls, below the diagonal correlations for boys.

Model Outcomes

In the next models, we tested the relation between the OXTR gene and state levels of loneliness. First, we tested the unconditional model without predictors. The intra-class correlation was .31 for girls and .33 for boys, indicating that 31-33% of the variation in state loneliness occurred at the individual level (Level 2). The variances in state loneliness were significant at the momentary assessment level (Level 1 variance boys = .28) and at the individual level (Level 2 variance boys = .13; Level 2 variance girls = .13). We tested whether the constrained model differed from the unconstrained model, which was not the case (Δχ² (2) = 1.44, p > .05), indicating that the unconditional model did not differ between boys and girls.

Subsequently, we entered the OXTR genotype as a predictor in the model (Level 2 model in Table 3). For boys, the relation between OXTR and state loneliness was not significant. In contrast, the relation between OXTR and state loneliness was significant for girls, in that A-allele carriers had higher levels of state loneliness than carriers of the GG genotype. The model in which the relation between OXTR and state loneliness was allowed to differ for boys and girls showed a significant improvement in model fit compared to the model in which this path was constrained to be equal across sex (Δχ² (1) = 5.77, p < .05).

Next, we examined the relations between negative company and state loneliness (Level 1 model in Table 3) and positive company and state loneliness (Level 1 model in Table 4). For both boys and girls, negative company was positively related to state loneliness, in that higher levels of negative company were related to higher levels of state loneliness. In addition, positive company was negatively related to state loneliness in both boys and girls, indicating that higher levels of positive company were related to lower levels of state loneliness. For both models, no differences were found between boys and girls (Δχ² (1) = 1.58, p > .05 for negative company; Δχ² (1) = 0.48, p > .05 for positive company).

In the next models, the interactions between the OXTR genotype and positive and negative company were examined (see Table 3 for negative company and Table 4 for positive company). No significant gene-environment interactions were found, and no sex differences were found (Δχ² (4) = 6.06, p > .05 for negative company; Δχ² (4) = 8.34, p > .05 for positive company). However, when we split the analyses for weekdays and weekend days; we did find a significant gene-environment interaction between OXTR and negative company for girls on weekend days only. When comparing the constrained model with the unconstrained model, it was found that this model did not significantly differ between boys and girls (Δχ² (4) = 5.62, p > .05). Further analyses showed that this interaction was present in the total sample (β = 1.5, SE = .05; p < .001). This finding indicated that adolescents found significant correlations between the OXTR genotype and state loneliness for girls, all subsequent analyses are tested in multigroup models, for boys and girls separately.

genotype and state loneliness and negative company, indicating that A carriers experienced slightly higher levels of state loneliness and negative company than girls with the GG genotype. State levels of loneliness were negatively correlated with positive company and positively correlated with negative company in both boys and girls. As we
### Table 3 Multi-Group Multilevel Models for Relations Between OXTR Genotype, Negative Company, and State Loneliness

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Boys All days</th>
<th>Boys Week days</th>
<th>Boys Weekend days</th>
<th>Girls All days</th>
<th>Girls Week days</th>
<th>Girls Weekend days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level-2</td>
<td>Level-1: random</td>
<td>Interaction</td>
<td>Level-2</td>
<td>Level-1: random</td>
<td>Interaction</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.31 (.07)***</td>
<td>1.23 (.03)***</td>
<td>1.27 (.07)***</td>
<td>1.19 (.05)***</td>
<td>1.25 (.03)***</td>
<td>1.29 (.03)***</td>
</tr>
<tr>
<td>Negative company</td>
<td>10 (.02)***</td>
<td>11 (.04)**</td>
<td>14 (.04)**</td>
<td>.02 (.04)</td>
<td>15 (.02)**</td>
<td>15 (.03)***</td>
</tr>
<tr>
<td>OXTR genotype</td>
<td>-.08 (.08)</td>
<td>-.06 (.08)</td>
<td>-.07 (.08)</td>
<td>-.01 (.08)</td>
<td>14 (.06)*</td>
<td>12 (.05)*</td>
</tr>
<tr>
<td>OXTR x Company</td>
<td>.00 (.05)</td>
<td>-.02 (.06)</td>
<td>.18 (.10)</td>
<td>1.22 (.03)**</td>
<td>1.26 (.03)***</td>
<td>1.14 (.03)***</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level-1 variance</td>
<td>.24 (.04)**</td>
<td>.14 (.03)***</td>
<td>.15 (.03)***</td>
<td>.17 (.03)***</td>
<td>.12 (.02)***</td>
<td>.28 (.03)***</td>
</tr>
<tr>
<td>Level-2 intercept variance</td>
<td>13 (.03)**</td>
<td>12 (.03)***</td>
<td>12 (.03)***</td>
<td>1.09 (.02)***</td>
<td>12 (.02)***</td>
<td>11 (.02)***</td>
</tr>
<tr>
<td>Level-2 slope variance</td>
<td>.04 (.01)**</td>
<td>.04 (.01)**</td>
<td>.04 (.01)**</td>
<td>.03 (.01)**</td>
<td>.03 (.01)**</td>
<td>.05 (.01)**</td>
</tr>
<tr>
<td>Model difference test</td>
<td>Chi square</td>
<td>5.77*</td>
<td>1.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Df</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: All observation-level variables were group-mean centered.

* p < .05. ** p < .01. *** p < .001.

### Table 4 Multi-Group Multilevel Models for Relations Between OXTR Genotype, Positive Company, and State Loneliness

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Boys All days</th>
<th>Boys Week days</th>
<th>Boys Weekend days</th>
<th>Girls All days</th>
<th>Girls Week days</th>
<th>Girls Weekend days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level-2</td>
<td>Level-1: random</td>
<td>Interaction</td>
<td>Level-2</td>
<td>Level-1: random</td>
<td>Interaction</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.31 (.07)***</td>
<td>1.23 (.03)***</td>
<td>1.27 (.07)***</td>
<td>1.19 (.05)***</td>
<td>1.25 (.03)***</td>
<td>1.29 (.03)***</td>
</tr>
<tr>
<td>Positive company</td>
<td>-.08 (.02)***</td>
<td>-.10 (.04)**</td>
<td>-.09 (.04)**</td>
<td>-.04 (.05)</td>
<td>-.02 (.02)**</td>
<td>-.08 (.02)**</td>
</tr>
<tr>
<td>OXTR genotype</td>
<td>-.08 (.08)</td>
<td>-.06 (.08)</td>
<td>-.07 (.08)</td>
<td>-.00 (.08)</td>
<td>14 (.06)*</td>
<td>12 (.05)*</td>
</tr>
<tr>
<td>OXTR x Company</td>
<td>.04 (.04)</td>
<td>.03 (.05)</td>
<td>.05 (.10)</td>
<td>-.03 (.03)</td>
<td>-.03 (.03)</td>
<td>-.07 (.06)†</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level-1 variance</td>
<td>.24 (.04)**</td>
<td>.14 (.02)***</td>
<td>.14 (.02)***</td>
<td>.16 (.03)***</td>
<td>.06 (.02)***</td>
<td>.28 (.03)***</td>
</tr>
<tr>
<td>Level-2 intercept variance</td>
<td>13 (.03)**</td>
<td>12 (.03)***</td>
<td>12 (.03)***</td>
<td>1.14 (.06)***</td>
<td>12 (.02)***</td>
<td>11 (.02)***</td>
</tr>
<tr>
<td>Level-2 slope variance</td>
<td>.01 (.00)**</td>
<td>.03 (.01)**</td>
<td>.04 (.01)**</td>
<td>.04 (.04)</td>
<td>.02 (.00)**</td>
<td>.02 (.00)**</td>
</tr>
<tr>
<td>Model difference test</td>
<td>Chi square</td>
<td>5.77*</td>
<td>0.48</td>
<td>8.34</td>
<td>7.09</td>
<td>7.39</td>
</tr>
<tr>
<td></td>
<td>Df</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: All observation-level variables were group-mean centered.

† < .07. * p < .05. ** p < .01. *** p < .001.
carrying an A-allele were more negatively affected by negative company on weekend days, in that they experienced higher levels of state loneliness when they perceived their company more negatively, than adolescents carrying the GG genotype (see Figure 2). For positive company, no significant gene-environment interactions were found, and no differences were found between boys and girls (see Table 4).7

![Figure 2 Relation between negative company and state loneliness on weekend days for girls, split for the OXTR genotype.](image)

### Discussion

The present study aimed to investigate relations between the OXTR genotype, perceptions of company, and state levels of loneliness. We found a significant relation with the OXTR rs53576 variant for girls, in that girls carrying an A allele had significantly higher levels of state loneliness than girls carrying the GG genotype. No significant interactions were found for perceptions of company and OXTR on state loneliness. However, when the analyses were split for week and weekend days, we did find significant gene-environment interactions. These results indicate that adolescents carrying an A allele may be more susceptible for negative environments, during weekend days.

### Main Effects OXTR Gene

We found a significant relation between state loneliness and the OXTR rs53576 variant in girls, in that girls carrying an A allele had significantly higher levels of state loneliness than girls carrying the GG genotype. These findings are in line with previous studies showing that the A allele is related to maladaptive outcomes (Bakermans-Kranenburg & van IJzendoorn, 2008; Kogan et al., 2011; Rodrigues et al., 2009; Saphire-Bernstein et al., 2011; Tost et al., 2010). Our findings are in contrast with results from van Roekel et al. (2013), who found the GG genotype to be related to stable levels of trait loneliness in adolescence. Importantly, as trait and state levels of loneliness are distinct phenotypes, it is difficult to compare these results.

### Gene-Environment Interactions

We found no significant gene-environment interactions between positive and negative company on state loneliness when we analyzed all days together. Yet, when we examined the relations for week and weekend days separately, we found significant interactions on weekend days only. These findings showed that adolescents carrying an A allele had higher levels of state loneliness when they perceived their company more negatively, than adolescents carrying the GG genotype (see Figure 2). For positive company, no significant gene-environment interactions were found, and no differences were found between boys and girls (see Table 4).7

---

7 In the present study, a dominant model for the A allele was assumed. Because of the small group of adolescents with the AA genotype (N = 30), it was not possible to examine differences in results between the three genotypes. However, we could examine whether the results would differ if we included only adolescents with GG genotypes versus adolescents with the AG genotype. No differences were found in these analyses, indicating that our results were not due to the small group of adolescents with the AA genotype.
negatively, whereas state loneliness in GG genotypes was not affected by negative company. This interaction is in line with a diathesis-stress model (e.g., Costello, Swendsen, Rose, & Dierker, 2008; Shanahan & Hofer, 2005), which states that dual risks, that is, carrying a ‘risk’ allele (i.e., the A allele) and experiencing negative environments (i.e., negative company) lead to most negative outcomes. Interestingly, we only found this gene-environment interaction for weekend days. A possible explanation may be that the company adolescents were in had a greater impact on them during weekends. In weekends, adolescents most often are free to choose who they want to spend their time with and how they want to spend their time, whereas on weekdays, they are less free to choose company. Therefore, their perceptions of the company they are with during weekends may have a greater impact on their feelings of loneliness. The importance of weekends is also accentuated by previous research, in which it was found that adolescents experience the highest levels of state loneliness when they are alone at weekend days, and more specifically, Friday and Saturday nights (Larson & Richards, 1998). The perceptions of company adolescents have during weekends may affect their loneliness levels more than the perceptions they have during week days.

To our knowledge, only three other studies found gene-environment interactions with the OXTR rs53576 variant on maternal sensitivity (Sturge-Apple et al., 2012), emotion dysregulation (Bradley et al., 2011), and physical health problems (Poulin & Holman, 2013). Two studies found that GG carriers were more susceptible for negative environments (i.e., interparental conflict and childhood maltreatment) (Bradley et al., 2011; Sturge-Apple et al., 2012), whereas the study on physical health problems found that A carriers were more susceptible to negative environments (Poulin & Holman, 2013). The results from Bradley et al. and Sturge-Apple et al. are in contrast with our findings, which showed that adolescents carrying an A allele were more negatively affected by their environment. Importantly, it is difficult to compare our findings to those of Bradley et al. and Sturge-Apple et al., because the outcome variables, the definitions of the environment, and the designs of the studies are not comparable. In our study, participants rated their environment when they were actually in it, whereas in the previous studies, participants had to report on their environment retrospectively. Therefore, it could be that GG carriers are more negatively affected by very negative, retrospectively rated environments, whereas A carriers are more affected by negative environments, at the moment that they experience those environments. These contradictory findings indicate that further research on gene-environment interactions with the OXTR gene is warranted.

Our findings indicate that adolescents carrying an A allele may be more susceptible to their environment. These effects were only found on a micro-level, which may explain why we found opposite results to the study of van Roekel et al. (2013). As adolescents carrying the GG genotype are less susceptible to their direct, real-life environment, which was found in the present study, their levels of trait loneliness may remain more stable, because these levels are not greatly affected by their environment, as was found in the study by van Roekel et al. (2013). In contrast, adolescents carrying an A allele may be more affected by their environment and therefore decrease in trait loneliness over time. As we did not have longitudinal data, we could not examine this assumption in our sample. Further research is necessary to explain the differences between the previous findings on trait loneliness and the present findings on state loneliness. A possible solution for this contradiction in findings may be to examine genetic effects and gene-environment interactions on both trait and state levels of loneliness in a longitudinal design, in which adolescents annually fill out trait loneliness questionnaires, and several ESM periods take place between successive annual waves. In that way, it is possible to examine genetic effects and gene-environment interactions on state and trait loneliness simultaneously.

In our additional analyses in which we checked whether the results for negative company differed between the two items (i.e., ‘I feel judged by this company’ and ‘I feel threatened by this company’), we showed that the significant gene-environment interaction with negative company on weekend days was only present for the ‘judged’ item, and not for the ‘threatened’ item. This indicates that A carriers respond more negatively to perceptions of judging company, and not to perceptions of threat. A possible explanation for this may be that in general, adolescents more often experience being judged by their company than being threatened by their company and therefore, differences between genotypes may become visible. However, further research is necessary to disentangle these different effects.

**Sex Differences**

Several explanations can be given for the sex differences in the findings of the present study. As was mentioned before, oxytocin receptors are affected by levels of estrogen, a sex hormone that is particularly present in females. Therefore, variation in this gene might be more relevant in girls. Importantly, sex differences are common in research on the OXTR rs53576 variant (Bakermans-Kranenburg & van Lijnden, 2004; Tost et al., 2010), but are not always found (Rodrigues et al., 2009; Saphire-Bernstein et al., 2011). Also, in this study the difference patterns were not consistent, warranting more research on sex differences in the effects of the OXTR gene.

**Strengths and Limitations**

One of the major strengths of the present study is that we examined gene-environment interactions in ‘the real world’. Although it is important that these results are replicated, our findings provide greater insight into the role of the OXTR gene in internalizing problems. In addition, because our measures are administered in daily life, our findings could provide more appropriate starting points for intervention and prevention than trait-level variables do.

A first limitation of the present study is that our sample size was relatively small, which might imply that we did not have sufficient power to find significant relations. However, our power increased as we measured our outcome variable as well as the environment...
multiple times, resulting in more reliable measures, which in turn increased power. This is also substantiated by the results from the power analyses, showing that we had enough power to detect a gene-environment interaction.

Second, because we only examined relations cross-sectionally, it is not possible to determine the direction of effects. It could be that the perceptions of company predict subsequent levels of state loneliness, but it is also possible that experiencing loneliness influences adolescents’ perceptions of company. Importantly, irrespective of the direction of effects, our results showed that the OXTR gene moderated these relations.

Third, mean levels of state loneliness were relatively low in our sample. Because the few studies that have examined state levels of loneliness did not report on mean levels, we could not compare our mean levels on state loneliness with other studies. However, mean levels of negative affect in other studies on early adolescents (i.e., anxiety, depressive feelings, and irritation; Schneiders et al., 2007) are comparable to our mean levels on state loneliness, which may indicate that these levels are not extraordinarily low.

Fourth, previous research showed that adolescents experienced more positive affect when they were with friends, compared to when they were with family (Larson, 1983), which indicates that the type of company adolescents are in affects their feelings. It is possible that in our sample, adolescents were more susceptible to a specific type of company. Splitting the analyses for these subgroups was not possible in the present study, as this would have resulted in very small groups. Future research could examine whether the type of company can explain why adolescents with an A allele were more affected by negative perceptions of company.

Fifth, currently we do not know what the functionality of the OXTR rs53576 variant is in terms of gene expression and actual oxytocin levels. Hence it could be that our findings are caused by another SNP that is in linkage disequilibrium with the rs53576 variant. Further research is necessary to examine the function of the OXTR rs53576 variant.

Conclusions

To conclude, the present study showed that girls carrying an A allele had higher levels of state loneliness than girls carrying the GG genotype. In addition, both boys and girls with an A allele were more negatively affected by negative company than boys and girls with the GG genotype, on weekend days only. Our findings highlight the importance of operationalizing the outcome variable and the environmental variables precisely and accurately, as we only find gene-environment interactions on weekend days.
Appendix 1

5-HTTLPR and DRD2 Genotypes in Relation to State Loneliness in Adolescence
Introduction

In Chapter 10, we showed that the OXTR gene is related to state levels of loneliness, and that it interacts with perceptions of company in predicting state loneliness. As previous studies in this dissertation have shown that the 5-HTTLPR gene and the DRD2 gene are related to trait levels of loneliness in adolescence (see Chapters 7 and 8, respectively), the aim of this appendix is to examine relations between these genes and state levels of loneliness.

First, we examined relations between the 5-HTTLPR and DRD2 genotypes and state levels of loneliness. We hypothesized that adolescents carrying the short allele of the 5-HTTLPR gene would have higher levels of state loneliness than adolescents carrying the long-long genotype. For the DRD2 gene, we hypothesized that adolescents carrying an A1 allele would have higher levels of state loneliness than adolescents carrying the A2A2 genotype.

Second, we examined whether these genotypes interact with positive and negative perceptions of company. We expected adolescents with the short allele of the 5-HTTLPR gene to be more affected by positive and negative perceptions of company, and that state levels of loneliness in adolescents with the long-long 5-HTTLPR genotype would not be affected by their perceptions of company. For the DRD2 gene, we expected A2A2 carriers to be affected by positive and negative company, and adolescents with at least one A1 allele not to be affected by perceptions of company.

Method

Participants

The total sample consisted of 303 participants who were recruited from four high schools (Mage = 14.18, SD = 0.54). Of the total group, 40% were boys and educational levels were equally distributed (25% preparatory secondary school for technical and vocational training, 35% attended preparatory secondary school for professional education, and 39% attended preparatory secondary school for university). The majority of the adolescents (97.1%) were born in The Netherlands and only 1.3% of the adolescents was not born in an European country.

Procedure

Data collection consisted of a baseline questionnaire, saliva collection, and momentary assessments. For a detailed description of the data collection procedures, see Chapter 2 and Chapter 10.
Measures

5-HTTLPR genotype. DNA was isolated from saliva using the Oragene system (DNA Genotek inc., Kanata, Ontario, Canada). Genotyping of the VNTR polymorphism in the SLCGA4 (5-HTT, SERT) gene was performed by simple sequence length analysis. PCR was on 50 ng genomic DNA using 10 pmol of forward primer (5'-GGGTTGCGGCTGCTGAATTGC-3') and 10 pmol reverse primer (5'-GAGGGACTGACGTGCAACACCAC-3'), 0.25 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen, Breda, The Netherlands) in a PCR buffer containing 0.3 M Tris-HCl (pH 8.3), 75 mM ammoniumsulfate and 7.5 mM MgCl₂. The cycling conditions for the polymerase chain reaction started with 5 min at 95°C, followed by 35 cycles of 1 min at 92°C, 1 min at the optimized annealing temperature (57.5°C, and 1 min 72°C, then followed by an extra 5 min 72°C. PCR products were analyzed on a 2% agarose gel. The amplification yielded distinct bands at 484 bp (short allele) and 528 bp (long allele).

The 5-HTT (rs25531) polymorphism was genotyped using Taqman analysis. A custom made Taqman Allelic Discrimination assay was ordered. This assay consisted of 2 primers (forward: CCGCTGCGGCTGCC, reverse: ATGCTGGAGGGGTGA) and 2 fluorescent probes (VIC-CTGCACCCCCAGCAT, FAM-CTGCACCCCCGGCAT, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 10 µl containing 20 ng of genomic DNA, 5 µl of Taqman Mastermix (2x; Applied Biosystems) and 0.25 µl of the Taqman assay (40x) and 2.75 µl of MilliQ. Each Amplification for the custom made Taqman Allelic Discrimination assay for the polymorphism rs25531 was performed by an initial denaturation at 95°C for 12 min, followed by 50 cycles of denaturation at 92°C for 15 seconds and annealing/extension at 60°C for 90 seconds, this was carried out on a 7900 Fast Real-Time PCR System. Genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems). Genotyping was performed in a CCOX-accredited laboratory at the Department of Human Genetics of the Radboud University Nijmegen Medical Centre in Nijmegen. Generally, 5% blanks as well as duplicates between plates were taken along as quality controls during genotyping. No deviations from Hardy-Weinberg equilibrium (HWE) were detected (p = .65). A dominant model was assumed, therefore we recoded the 5-HTTLPR genotype into 1 for short/Lg carriers and 0 for LaLa genotypes.

DRD2 genotype. DRD2 genotyping. The DRD2 Taq A C>T polymorphism was genotyped using Taqman analysis (assay ID: Taqman assay_C___7486676_10; reporter 1: VIC-A-allele, reverse assay, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 10 ul containing 10 ng of genomic DNA, 5 ul of Taqman Mastermix (2x; Applied Biosystems), 0.125 ul of the Taqman assay, and 3.875 ul of H₂O. Genotyping was performed on a 7500 Fast Real-Time PCR System and genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems).

To investigate the random genotyping error rate, the lab included 5 duplicate DNA samples per 96-well plate, which were 100% consistent. In addition, 4 blanks were included in each plate, which were required to be negative. No deviations from HWE were detected (p = .54). To maximize the power of the analyses, DRD2 genotype was dummy-coded into 0 (A2A2) and 1 (A1A2 and A1A1).

State levels of loneliness. State levels of loneliness were measured with four items; I feel lonely, abandoned, isolated, and rejected. At each assessment, adolescents rated to what extent they experienced these emotions on a scale ranging from not at all (1) to very much (7). Cronbach’s alpha was calculated for each momentary assessment separately, and then averaged over all momentary assessments, which resulted in an alpha of .75.

Perceptions of company. When adolescents were not alone, they rated how they perceived their company on a scale ranging from not at all (1) to very much (7). Positive company consisted of the items ‘I feel accepted by this company’ and ‘I feel comfortable in this company’. Negative company was measured with the items ‘I feel threatened by this company’ and ‘I feel judged by this company’.

Results

Descriptive Statistics

Of the total sample, 263 adolescents had data on the 5-HTTLPR genotype. Of this group, 68 adolescents carried the LaLa genotype, and 195 adolescents carried at least one S or Lg allele. For the DRD2 gene 266 adolescents were included, of which 170 adolescents carried the A2A2 genotype, and 96 adolescents carried the A1A1 or A1A2 genotype. In order to calculate descriptive statistics, momentary assessment mean scores were aggregated over the six days, resulting in a mean score for each individual. Mean state levels of loneliness and negative perceptions of company were relatively low, compared to the range (i.e. 1-7, see Table 1), whereas positive company was relatively high. Higher levels of state loneliness were related to higher levels of negative company, and lower levels of positive company. No relations were found between the DRD2 and 5-HTTLPR genotypes and state loneliness. Both genotypes were correlated with positive and negative company, in that adolescents carrying an A1 allele of the DRD2 gene had higher levels of negative company, and marginally significant lower levels of positive company. Adolescents carrying an S or Lg allele of the 5-HTTLPR gene experienced lower levels of negative company and higher levels of positive company. Sex and age were not correlated with any of the model variables.

Model Results

First, we examined relations between the 5-HTTLPR genotype and state loneliness (Table 2). For boys, we found a significant relation between the 5-HTTLPR genotype and state levels of loneliness. However, when we compared the model in which the paths were constrained to be equal across sex with the model in which the paths were allowed
to vary across sex, no significant differences were found ($\Delta \chi^2 (1) = 2.04, p > .05$), indicating that the paths did not differ between boys and girls. In the total sample, the relation between 5-HTTLPR and state loneliness was not significant ($B = -.11, SE = .06, p > .05$). No significant gene-environment interaction was found between 5-HTTLPR and negative company (see Table 2). The gene-environment interaction between positive company and the 5-HTTLPR genotype was significant for girls only. However, when we compared the constrained model with the unconstrained model, no significant differences were found ($\Delta \chi^2 (4) = 6.52, p > .05$), indicating that the model did not differ between boys and girls. In the total sample, the interaction remained significant ($B = .08, SE = .03, p < .01$), showing that adolescents carrying the S/Lg allele were not affected by their positive perceptions of company, whereas adolescents carrying the LaLa genotype had lower levels of state loneliness when they perceived their company positively and higher state loneliness when they perceived their company less positively (see Figure 1).

Second, relations between the DRD2 genotype and state loneliness were examined (Table 3). For both boys and girls, no significant relations were found. Next, we examined the cross-level interactions between the DRD2 genotype and negative and positive company. No significant gene-environment interactions were found, for boys nor girls.

---

Note: * $p < .05$, ** $p < .01$, *** $p < .001$. 0 = boy; 1 = girl. 0 = A2A2, 1 = A1A1/A1A2. 0 = LaLa, 1 = S/La/LgLg/LgLl.

---

Table 1: Correlations Between Model Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>M (SD)</th>
<th>N</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
<th>5.</th>
<th>6.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age</td>
<td>14.18 (0.54)</td>
<td>266</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Sex 0</td>
<td>0.59 (0.49)</td>
<td>266</td>
<td>-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. DRD22</td>
<td>0.36 (0.48)</td>
<td>266</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. 5-HTTLPR</td>
<td>0.74 (0.44)</td>
<td>263</td>
<td>11</td>
<td>-10</td>
<td>-06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. State loneliness</td>
<td>1.30 (0.37)</td>
<td>266</td>
<td>-02</td>
<td>08</td>
<td>12</td>
<td>-16*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Negative company</td>
<td>1.51 (0.52)</td>
<td>266</td>
<td>-05</td>
<td>06</td>
<td>16*</td>
<td>-15*</td>
<td>65***</td>
<td>-</td>
</tr>
<tr>
<td>7. Positive company</td>
<td>6.19 (0.58)</td>
<td>266</td>
<td>07</td>
<td>-03</td>
<td>-10</td>
<td>13*</td>
<td>-56***</td>
<td>-7***</td>
</tr>
</tbody>
</table>

Table 2: Multi-Group Multilevel Models for Relations Between 5-HTTLPR Genotype, Negative and Positive Company, and State Loneliness

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Level-2 Interaction</th>
<th>Level-2 Interaction</th>
<th>Level-2 Interaction</th>
<th>Level-2 Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.23 (.04)**</td>
<td>1.20 (.04)**</td>
<td>1.29 (.04)**</td>
<td>1.25 (.03)**</td>
</tr>
<tr>
<td>Company</td>
<td>.13 (.04)**</td>
<td>-.18 (.03)**</td>
<td>.14 (.04)**</td>
<td>-.20 (.03)**</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>-.21 (.09)*</td>
<td>-.21 (.09)*</td>
<td>-.04 (.08)</td>
<td>-.07 (.07)</td>
</tr>
<tr>
<td>5-HTTLPR x Company</td>
<td>-.03 (.06)</td>
<td>-.08 (.05)</td>
<td>.05 (.05)</td>
<td>.10 (.04)*</td>
</tr>
</tbody>
</table>

Model difference test
| Chi square | 2.04                | 4.75                | 2.04                | 4.75                |
| Df         | 1                   | 4                   | 1                   | 4                   |

Table 3: Multi-Group Multilevel Models for Relations Between DRD2 Genotype, Negative and Positive Company, and State Loneliness

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Level-2 Interaction</th>
<th>Level-2 Interaction</th>
<th>Level-2 Interaction</th>
<th>Level-2 Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.23 (.04)**</td>
<td>1.20 (.04)**</td>
<td>1.29 (.04)**</td>
<td>1.25 (.03)**</td>
</tr>
<tr>
<td>Company</td>
<td>.04 (.01)**</td>
<td>-.08 (.07)</td>
<td>.10 (.06)</td>
<td>-.16 (.06)</td>
</tr>
<tr>
<td>DRD2 genotype</td>
<td>.07 (.06)</td>
<td>.08 (.07)</td>
<td>.15 (.08)</td>
<td>.08 (.08)</td>
</tr>
<tr>
<td>DRD2 x Company</td>
<td>-.09 (.04)</td>
<td>.02 (.04)</td>
<td>.04 (.04)</td>
<td>.06 (.04)</td>
</tr>
</tbody>
</table>

Model difference test
| Chi square | 2.04                | 4.75                | 2.04                | 4.75                |
| Df         | 1                   | 4                   | 1                   | 4                   |

---

8 In Chapter 10, we found that the gene-environment interaction with the OXTR gene was only significant on weekend days. We checked whether the results on 5-HTTLPR, DRD2 differed if we would analyzer week and weekend days separately. This was not the case.
difference in findings may be due to differences in the providers of support, as we measured only parental support in the longitudinal study, and perceptions of all types of company in the present study. Further research is necessary to elucidate the differences in findings between trait and state loneliness.

Discussion

The aim of this appendix was to examine relations between the DRD2 and 5-HTTLPR genotype and state levels of loneliness. For the DRD2 genotype, we found no relation between the genotype and state loneliness, and no gene-environment interactions were significant, for boys nor girls. For the 5-HTTLPR genotype, a gene-environment interaction was found, in that adolescents carrying the LaLa genotype benefited from higher levels of positive company, whereas adolescents carrying a S/Lg allele were not affected by their positive perceptions of company.

The findings for 5-HTTLPR are in contrast with our expectations and the gene-environment findings regarding the 5-HTTLPR gene and trait loneliness in Chapter 7. In this Appendix, we found that LaLa carriers were more affected by positive company, whereas in the longitudinal study we found that short allele carriers were more affected by their environment than long-long genotypes. We do not have an explanation why the results on state loneliness differ from previous results on trait loneliness and results on the 5-HTTLPR gene in relation other negative outcomes (e.g., depression; Clarke et al., 2010). Further research on the role of 5HTTLPR in state and trait loneliness is necessary in order to explain this difference in findings.

Regarding the DRD2 genotype, no significant results were found in relation to state loneliness. This was in contrast with our expectations and our previous results, as we showed in the longitudinal study in Chapter 8 that adolescents with the A2A2 genotype were more susceptible to parental support. A possible explanation for this difference in findings may be that adolescents with the A2A2 genotype are susceptible to more general levels of support as we measured in the longitudinal study, whereas they show less pronounced responses to immediate, real-life experiences of support. Further, the
Chapter 11

General Discussion
Summary of Main Findings

Part I: Daily Life Processes

Chapter 2
• State loneliness was highest when adolescents were alone, compared to being with company.
• When adolescents were with company, state loneliness was highest at school and with classmates and lowest at home and other locations, with family or friends.
• Boys and girls showed a relief effect when they entered the company of friends after being alone.
• Only girls showed a spill-over effect of solitude when they entered the company of family after a period of solitude.

Chapter 3
• Evidence was found for the differential reactivity hypothesis, in that high lonely adolescents in all samples responded more negatively to situations alone (i.e., had higher levels of state loneliness), compared to low lonely adolescents.
• High lonely adolescents benefited more from being with company (intimate company specifically) in that their levels of state loneliness decreased more in company, compared to low lonely adolescents.
• Early adolescents with high levels of trait loneliness did not show differences in state loneliness between situations alone and situations with non-intimate company, compared to early adolescents with low levels of loneliness.

Chapter 4 and 5
• In early and late adolescents samples, trait lonely adolescents had more negative and less positive perceptions of others than low lonely adolescents.
• Support was found for hypersensitivity to social threat, in that lonely adolescents showed stronger negative responses to higher levels of negative company than low lonely adolescents.
• Results regarding hyposensitivity to social reward were in opposite direction to our hypothesis; lonely adolescents showed stronger (and not lower) positive responses to higher levels of positive company than non-lonely adolescents.

Chapter 6
• Adolescents experienced peaks in negative company most often when they were with classmates (versus family, friends and others), during week days (versus weekend days), and in the morning (versus afternoon and evening).
• Some adolescents never experienced peaks in negative company (especially boys), and some adolescents were not affected by peaks in negative company in their mood levels (especially boys and adolescents low in loneliness).
• Lonely adolescents experienced higher peaks in negative company, but not more peaks, and responded more negatively to peaks than non-lonely adolescents.
Part II: Genetic influences
Chapter 7
- Loneliness is highest in early adolescence and decreases throughout adolescence
- Short allele carriers of the 5-HTTLPR gene remain stable in loneliness throughout adolescence, whereas long-long genotypes decreased
- Short allele carriers (5-HTTLPR) were more susceptible to maternal support only

Chapter 8
- The DRD2 genotype was not related to the onset or development of loneliness
- A2A2 genotypes were more susceptible to parental support

Chapter 9
- Girls carrying the GG genotype of the OXTR gene remained stable in loneliness throughout adolescence, whereas girls carrying an A allele decreased
- No gene-environment interactions were found between parental support and the OXTR gene
- A gene-gene interaction was found, in that adolescents who had an A1 allele for the DRD2 gene and had the GG genotype for the OXTR gene showed stable levels of loneliness over time, whereas other genotype combinations decreased in loneliness

Chapter 10
- Girls carrying an A allele of the OXTR gene had higher levels of state loneliness than girls carrying the GG genotype
- Both boys and girls with an A allele were more affected by negative perceptions of company than GG carriers, on weekend days only

Part I: Daily Life Processes

Reflection on Main Findings
Relevance of Social Contexts. Previous research has revealed that the relevance of social contexts changes during adolescence, as adolescents for example spend less time with family, and more time with friends (Larson & Richards, 1991). Furthermore, the emotions experienced with family become less positive, whereas the emotions experienced with friends become more positive (Larson, 1983). A recent evolutionary theory, Social Baseline Theory (Beckes & Coan, 2011), states that the proximity of others represents a baseline state of relative calm, because being with others allows for distribution of risks when presented with outside threats (e.g., physical threats), and enables load sharing (e.g., caring for each other, sharing goals and resources). Based on this theory and previous findings, we hypothesized that adolescents would experience being with intimate company, such as family and friends, more positively (i.e., higher levels of positive affect, lower levels of negative affect and state loneliness) compared to being alone. Further, being with non-intimate company, for example classmates, was expected to be a more positive experience than being alone, but slightly more negative than being with intimate others, as the amount of load sharing and risk distribution may be lower with non-intimate company. The present dissertation was the first to examine differences in state loneliness between different social contexts (i.e., different types of company).

As expected, our results showed that the social contexts adolescents were in affected their levels of state loneliness. In general, being alone was the most negative situation for both early and late adolescents in that adolescents experienced highest levels of state loneliness when alone (see Chapter 2 and 3), independent of the location (i.e., home, school, or public places) in which adolescents were alone (Chapter 2). Although Larson (Larson, 1990, 1997; Larson & Csikszentmihalyi, 1980) has found similar results in his studies (i.e., being alone was related to higher negative affect and state loneliness, and lower positive affect), he argued that being alone also has beneficial effects, because a moderate time spent alone (i.e., 20% - 35% of waking hours) was related to better psychological adjustment (Larson & Csikszentmihalyi, 1980) and adolescents showed a positive after-effect of solitude, in that they experienced higher levels of positive affect when they entered the company of others after a period of solitude. The latter was also found for state loneliness in the present thesis, in that adolescents experienced lower levels of state loneliness when they entered the company of friends, after being alone. It may be questionable however whether this should be interpreted as a positive after-effect of solitude or as a relief effect, in that adolescents are relieved that they are not alone anymore and have entered the company of friends. We feel that the latter may be more logical, as adolescents in general experience solitude to be negative, and not positive. Further, when this pattern of findings would reflect a positive after-effect, we should have found similar effects for all types of company, but we only found this effect for entering the company of friends. For girls, we even found a spillover effect of solitude when they entered the company of family, showing that being alone had lasting negative effects on state loneliness. In addition to this, a relief effect would be in line with the Social Baseline Theory (Beckes & Coan, 2011), because according to this theory, being alone may be a particularly negative situation compared to being with others, as it requires more emotion regulation efforts and a higher state of vigilance for potential threats because there are no others around to protect them and help them regulate the negative emotions of feeling less safe. Therefore, as being with others is hypothesized to represent a baseline state of calmness and safety, entering the company of others would lead to a clear increase in positive emotions, or in our case, a steep decrease in state loneliness, which can be interpreted as a relief effect.

Nevertheless, we do agree with Larson that solitude can have its benefits as well. According to several researchers (Larson, 1990, 1997; Long & Avrell, 2003), one of the most important factors that determines whether solitude is experienced positively or negatively, is the amount of control a person has over the situation. In other words, when adolescents chose to be alone, solitude may not have been a negative experience, whereas situations in which adolescents did not want to be alone, may have been experienced negatively.
Furthermore, solitude was also experienced less negatively when it was used constructively, for example, when adolescents used solitude to concentrate or focus on homework, or to reflect on the self (e.g., Larson, 1997; Larson & Csikszentmihalyi, 1980; Larson et al., 1982). Hence, we might have found different results if we would have examined how adolescents spent their time alone and if we would have known whether adolescents chose to be alone, or were alone because of certain circumstances. In addition, we did not know whether adolescents, while being alone, still had contact with others by means of other media (i.e., texting, calling, chatting). These virtual activities likely have influenced our results, as most young adolescents in the Netherlands have a smartphone (i.e., 80% of 13-year olds) and use social media to keep in touch with their friends (Stichting Mijn Kind Online & Kennisnet, 2013). Hence, to obtain a more complete picture of the effects solitude has on adolescents, further research is necessary in which it is examined whether adolescents choose to be alone and what activities adolescents carry out when they are alone. We will discuss these suggestions for further research in more detail further in this General Discussion.

When comparing the situations in which early adolescents were with others, it was found that they experienced highest levels of state loneliness when they were with classmates, compared to being with family, friends, or others. For late adolescents, similar results were found, in that the highest levels of state loneliness occurred with non-intimate company (e.g., classmates, teammates, etc.). This is not surprising, because classmates are peers that adolescents often do not voluntarily choose to be with, and hence the relationships adolescents have with classmates are likely to be of lower quality compared to the relationships they have with their friends or family. Therefore, adolescents may feel more lonely when they are in company that they do not necessarily have a good relationship with (in this case, classmates). These findings are also in line with Social Baseline Theory, in which it is stated that intimate company has greater advantages than non-intimate company, as only intimate company may provide the opportunity for load sharing and risk distribution. Therefore, being with intimate company would be the safest situation for adolescents, and therefore they may feel least lonely in those situations. These findings may be recognizable to us all, as we all as adolescents likely have experienced a situation in which we had to enter a cafeteria, school yard or party filled with other adolescents who were watching and judging you (or at least you thought so). Facing such situations alone could be an uncomfortable or threatening experience, whereas being in a group of friends in such a situation could provide a safe base which made you feel more secure and protected from those presumed judging others. Because adolescents spent a great amount of their time with classmates (i.e., around 26% of their time, compared to 11% spent with friends, and 21% with family; see Chapter 2), our findings indicate that adolescents are a large amount of time in company that increases feelings of loneliness. We could therefore conclude that being with classmates is a specific stressful time for adolescents, which should maybe be avoided. However, as other studies have shown that adolescents in general experience more stress and respond more negatively to rejection than pre-adolescents (Silk et al., 2012; Stroud et al., 2009), these heightened negative experiences with classmates may be normative in adolescence. Hence, further research is needed to investigate whether these negative experiences with classmates are normative, or are related to decreases in wellbeing.

Some further differences were found between the two types of intimate company: family and friends. Even though state levels of loneliness did not differ between situations with family or friends, we did find different effects when we examined the temporal effects of these social contexts on state loneliness. Both boys and girls showed a relief effect when they entered the company of friends after a period of solitude. For girls, opposite results were found for situations with family. When girls were alone at T1 and with family at T2, they had higher levels of loneliness than in situations in which they were with family at both time points, whereas for boys, no differences were found between those situations. A possible explanation for these sex differences may be that for boys, solitude is more normative than for girls, which is reflected in the findings in Chapter 2 that indicated that boys spend more time alone than girls. Therefore, they may not show a negative after effect once they have entered the company of family. A reason why we found a relief effect for friends only could be that adolescents often actively choose to be with friends, whereas family is normally available when adolescents are at home. Because of this, the time adolescents spent with friends is more likely to consist of social activities and include more higher quality social interactions (Larson, 1983) than the time spent with family. This could also explain our finding in Chapter 4 that adolescents experience higher levels of positive affect with friends, compared to family. Further research should measure the type of activities adolescents engage in with different types of company and the intensity of contact with their company to elucidate and explain these differences in findings between family and friends. On the other hand, the time spent with friends may not be solely positive, as our results in Chapter 6 showed that adolescents were more likely to experience peaks in negative company in situations with friends, compared to family. These findings may indicate that being with friends, which is a positive and non-lonely situation, may sometimes be experienced as negative, as adolescents more often experience extreme levels of negative social experiences with friends than with family. This may not be surprising, as research has shown that conflicts are present in friendships as well, and conflicts within a friendship may even have positive effects (Laursen, 1993). Further, as adolescents define themselves mainly in terms of their social relationships (e.g., Parkhurst & Hopmeyer, 1999) and are particularly sensitive to peer rejection (Silk et al., 2012), adolescents may be focused on picking up possible rejection cues from their friends, and therefore may more often experience peaks in negative company with their friends, compared to family.

Importantly, we did not examine how close adolescents were with their company. Future research can extend our findings on more objective social contexts (i.e., being with different types of company) by adding measures of closeness in order to disentangle the
relations with state loneliness in more detail. In addition to this, we did not examine to what extent adolescents were actively involved with the company they were in, whereas this may have had an effect on state loneliness. For example, when adolescents would be actively interacting with their company, levels of state loneliness may have been lower compared to situations in which others were only present, and no interactions occurred. Future research should examine these relations in more detail.

**Trait loneliness.** A further aim of the present thesis was to examine whether trait loneliness moderated the relations between social contexts and state loneliness. This is an important addition to the literature, as no studies have yet examined how and when trait lonely adolescents experience state levels of loneliness in their daily lives. In Chapter 3, we found support for the differential reactivity hypothesis (Cacioppo et al., 2003), in that trait lonely adolescents showed greater differences in state loneliness between situations alone and with intimate company than non-lonely adolescents. This finding can be interpreted in two directions; lonely adolescents may respond more negatively to being alone, or they may benefit more from being with intimate company than non-lonely adolescents. In addition, we found in Chapter 4 that trait lonely adolescents experienced higher levels of negative affect with classmates, compared to family. These findings combined indicate that compared to non-lonely adolescents, lonely adolescents thrive best in situations with intimate company such as family and worse when they are alone or with non-intimate company, such as classmates. This may be explained in terms of the Social Baseline Theory as well, as this theory implies that situations are perceived as more threatening when alone, a bit less threatening when with non-intimate others, and the least threatening when with intimate others. As lonely individuals perceive more threats than non-lonely people (Hawkley et al., 2003), this could explain why they respond more negatively to situations alone and more positively to situations with intimate others, because for them, the safe haven that intimate others can provide is more important as they perceive more risks than non-lonely individuals. Based on this evolutionary point of view, being with intimate others such as family is a particularly safe place for lonely adolescents, in which they do not have to worry about other people’s perceptions of them or more direct physical threats, as their family is present to help protect them.

Importantly, the results on trait and state loneliness were remarkably similar in early and late adolescents, and Dutch and American adolescents. There was one important difference, however, early adolescents with high levels of trait loneliness showed no difference in state loneliness between being alone and being with non-intimate company, whereas lonely late adolescents had lower levels of state loneliness when with non-intimate company. This indicated that lonely early adolescents did not benefit from being with non-intimate company. A possible explanation for this difference may be that non-intimate company, who were most often classmates in the early adolescent sample, do not protect from threats outside of the social group, because they may represent a threatening context themselves for lonely adolescents. In this way, being with non-intimate company does not provide a safer situation for adolescents compared to being alone, as classmates could be perceived by lonely adolescents as particularly threatening and rejecting, especially in early adolescence.

**Socio-Cognitive Model.** In Chapters 4 and 5, we further examined perceptions of threat of lonely adolescents, by investigating two characteristics of the socio-cognitive model (Cacioppo & Hawkley, 2009), that is, hypersensitivity to social threat and hyposensitivity to social reward, in the daily lives of early and late adolescents. We did so by investigating the affective responses of low and high lonely adolescents to positive and negative perceptions of company. Based on this model, we hypothesized that lonely adolescents would respond more negatively to negative company, and less positively to positive company. In both early and late adolescents, support was found for hypersensitivity to social threat, in that high lonely adolescents experienced greater increases in negative affect and greater decreases in positive affect when they perceived their company as more negative. For hyposensitivity to social reward, opposite results were found to what was theoretically expected, in that adolescents high in loneliness decreased more in negative affect when they perceived their company more positively. In sum, our findings point to hyposensitivity to social threat and social reward. Several explanations can be given for these results.

First, our findings may point to a general sensitivity to the environment in high lonely adolescents. This would be in line with evolutionary theory, which implies that loneliness may function as a motivational state (Cacioppo, Hawkley, et al., 2006). In this view, loneliness serves as a signal that something is wrong (i.e., social pain), which in turn makes individuals more sensitive to their environment, so that further threats can be avoided and positive signals are picked up in order to restore social relationships. Importantly, this heightened sensitivity to the environment may be especially present in temporary experiences of loneliness. It could be that experiencing transient feelings of loneliness increases sensitivity to both positive and negative environments, but when social relationships are not restored and feelings of loneliness become chronic, individuals may eventually become less sensitive to positive environments. Thus, when levels of loneliness become chronic, adolescents may get trapped in a vicious circle of negativity, as was proposed by Cacioppo et al. (2009), and hyposensitivity to social reward may come into play. As we did not measure the stability of trait loneliness in the ESM study, it was not possible to examine this potential explanation. However, future research should examine these characteristics in a longitudinal design, which would open the possibility to investigate whether initially temporal feelings of loneliness serve as a motivational state leading to hyposensitivity to both positive and negative environments. Further, it would be possible to examine whether this hyposensitivity turns into hyposensitivity to social reward when feelings of loneliness become chronic.

Second, the differences in findings between previous research (Cacioppo et al., 2009) and the studies presented in the present dissertation regarding sensitivity to social reward
may be explained by differences in measures. We measured subjective experiences of adolescents by examining their responses to positive perceptions of company, whereas the study that found support for hypersensitivity (Cacioppo et al., 2009) had more objective, but non-personalized measures of the social stimuli that were the same for all participants (i.e., positive pictures of social situations). It may be that lonely adolescents show less brain activation in reward areas in response to general social stimuli that may not be relevant to them (Cacioppo et al., 2009), whereas they are rewarded by personally salient social stimuli, that is, the company they are in. Importantly, as we measured only their perceptions of the company they were in, and we also found that lonely adolescents perceived their company more negatively and less positively, it may also be that once they experience their company as positive (which may not often be the case), they are more rewarded by those positive perceptions than non-lonely adolescents.

Further, because we used subjective measures, we do not know whether the high levels of negative company and low levels of positive company in lonely adolescents may be realistic perceptions, because their environments are indeed objectively more negative than those of non-lonely adolescents. It has been shown that there may be subgroups of lonely children, in that some children experienced loneliness whereas more objective indications showed that they were accepted by their peers, suggesting that loneliness was only based on their perception and was not substantiated by objective data (i.e., they perceived themselves as socially isolated, whereas they were not objectively socially isolated). On the other hand, some children experienced loneliness and were indeed found to be rejected by their peers, indicating that they felt lonely because they were indeed socially isolated (Qualter & Munn, 2002). In our study, we did not have data on the intersubjective and objective social world of our sample, and hence we cannot conclude whether only the perception of lonely adolescents was more negative, or that their social worlds are truly more negative than those of non-lonely adolescents. Although we did not distinguish between those possible subtypes of lonely adolescents, these subtypes may have different characteristics, and hence different types of interventions will be necessary to decrease their levels of loneliness.

According to Pickett and Gardner’s model of belonging regulation (Gardner et al., 2005; Pickett & Gardner, 2005), there are several steps in the belonging regulation process. First, individuals have to experience a lack of belonging, which is signaled to the self by negative affect, for example (i.e., the experience of loneliness). Second, the social monitoring system is activated, which monitors the environment for social cues and opportunities for social connection. Third and finally, these environmental cues are used to initiate social interactions in order to restore feelings of belonging. Hence, this final step includes the behavior that individuals engage in to enhance social inclusion. Although these steps can lead to social inclusion and reductions in loneliness, ongoing feelings of loneliness indicate that some adolescents fail in completing these steps. In the case of the two loneliness subtypes that were described before (i.e., high lonely and rejected versus high lonely and not rejected), we hypothesize that these subtypes fail in different phases of the regulatory model. For the subgroup of adolescents that are also rejected by their peers (i.e., objective social isolation), there may be problems with the final stage of the regulatory model particularly, that is, the behavioral stage in which they have to act to regain social inclusion. Previous research has shown that this subgroup has extremely high levels of both internalizing and externalizing problems (Qualter & Munn, 2002), and importantly, that this group often failed in their attempts to interact with other children. These findings indicate that this particular subgroup of adolescents may lack the social skills to re-establish their social relationships.

For the adolescents who are not objectively isolated, the experience of loneliness may be particularly due to errors that occur in the social monitoring phase. These adolescents may fail at correctly recognizing social cues, and may particularly be hypersensitive to social threat. On the other hand, as these adolescents are accepted by their peers, it is likely that they do not have a lack of social skills and are able to show socially acceptable behaviors. Hence, in this subgroup, it is merely the (negative) perception of adolescents and the cognitive attributions adolescents make rather than a lack of social skills that cause feelings of loneliness. Further, there may also be differences between the subgroups in the expectations adolescents have of their social relationships, which is part of the first step of the regulatory model (i.e., experiencing a lack of belonging). The adolescents who are not objectively isolated may hold unrealistic expectations about what the quality or quantity of their social relationships should be, which may cause them to feel lonely. This is important knowledge for intervention efforts, as these different subtypes need different therapeutic approaches. For example, in the objectively and subjectively isolated group, social skills training may be necessary, whereas in the only subjectively isolated group, cognitive behavioral therapy could be used to change their maladaptive cognitions.

In the present thesis, we did not differentiate between these subtypes of lonely individuals. It would seem plausible to hypothesize that the lonely and accepted subgroup is characterized by hypersensitivity to social threat and hyposensitivity to social reward, as this group may have difficulties with recognizing social cues. The lonely and isolated subgroup may not show these characteristics and only fail at the behavioral phase of the belonging regulation model. However, only a few studies have examined these different subtypes in lonely individuals and no studies have examined the social regulatory model in relation to those subtypes. Further research is necessary to support our hypotheses.

**Differential Reactivity and Exposure Hypotheses.** Based on the chapters in Part I of the present thesis, we can conclude that the differential reactivity hypothesis is confirmed, in that lonely adolescents responded differently to certain real-life environments and situations than non-lonely adolescents. To some extent, this differential reactivity was negative in nature, because lonely adolescents responded more negatively to negative company, to being alone, and to being with non-intimate others. However, we also found
some positive differences, in that lonely adolescents benefited more from positive company and being with intimate company. As was mentioned before, these findings may provide evidence for the evolutionary model, in which loneliness is viewed as a motivational state that enhances sensitivity to the social environment so that social relationships can be re-established (Cacioppo et al., 2003).

This differentially reactivity is in line with research by Gardner and Pickett on the social regulatory model (Gardner et al., 2005), in which they found that lonely adolescents had increased social memory and were better able to detect social cues than non-lonely adolescents. These findings implied that the social monitoring system was not malfunctioning in lonely adolescents, and may even work better in lonely adolescents, compared to non-lonely adolescents. Although the results in this thesis showed that trait lonely adolescents experience different affective responses to their environment compared to non-lonely adolescents, we do not know how adolescents in turn respond to this heightened sensitivity in their behavior (i.e., the behavioral phase of the social regulatory model) and how this differential reactivity affects their future levels of trait loneliness. It could be that the heightened sensitivity the environment in the end reduces their feelings of loneliness, because they respond to this sensitivity by showing positive social behavior which restores their social relations. But it may also be the other way around, for example, when their increased sensitivity to negative environments makes them behave more negatively (i.e., a negative regulatory loop, as suggested by Cacioppo & Hawkley, 2009), which further increases social exclusion and hence loneliness. Therefore, further research is needed to examine how this differential reactivity may affect actual behavior and future trait loneliness levels (this will be discussed in more detail in the directions for further research). In addition, we do not know whether the sensitivity to positive social environments is related to the sensitivity to negative social environments. It may be that we actually are looking at different subtypes of lonely people, one subtype that is more sensitive to positive environments, which may in the end decrease loneliness levels, and one subtype that is more sensitive to negative environments, in which loneliness levels are sustained or even increased. Future research should examine the relations between these two characteristics.

Regarding the differential exposure hypothesis, we found little evidence that lonely adolescents are indeed exposed to more negative environments than non-lonely adolescents. Only in the late Dutch adolescent sample, a small correlation was found between trait loneliness and the time spent with intimate company. In the present dissertation, we examined differential exposure by looking at the time spent in different social contexts. These were ‘objective’ contexts, as adolescents filled out who their company was, without a subjective connotation. Most other studies examining differential exposure have looked at the number of stressors people experience, which were often subjectively rated by the participant (e.g., Bolger & Zuckerman, 1995; Cacioppo et al., 2003). In this way, a heightened number of stressors may not represent differential exposure, but may as well be differential reactivity, as certain individuals may perceive more stressors than others, which does not necessarily reflect reality. Hence, it is important that further research examining the differential exposure hypothesis uses objective measures of the environment, so that a clear distinction can be made between heightened (objective) exposure to stressors versus heightened (subjective) reactivity to stressors.

Specificity of Effects. Previous research has shown that loneliness is highly correlated with depression (e.g., Vanhalst, Klimstra, et al., 2012), which was also found in the present dissertation (e.g., r = .53, see for example Chapter 4). Because we wanted to examine whether our results were specific for loneliness and not due to high correlations between loneliness and depression, we controlled for depressive feelings in most chapters. Overall, the effects that were found for trait loneliness did not change when we controlled for depressive feelings, showing that our main findings were specific for loneliness. However, there were some differences in specific findings when we controlled for depressive feelings in the different chapters. For example, in Chapter 4 and 5, in which we tested the characteristics of the socio-cognitive model in early and late adolescents, we found that relations between trait loneliness, perceptions of company, and affect changed when we controlled for depressive feelings. In early adolescents, the main affect of loneliness on negative affect became non-significant when depressive feelings were entered in the model, showing that depressive feelings were more strongly related to negative affect in this sample. In late adolescents, this was not the case. Loneliness remained a significant predictor for negative affect when we controlled for depressive feelings, and the relation with negative affect was even stronger for loneliness, compared to depressive feelings. This shows that there may be an age difference in the effects of loneliness and depressive feelings on negative affect. In early adolescents, depressive feelings seem to be more important, whereas in late adolescence, feelings of loneliness are more strongly related to negative affect. These findings highlight the importance of examining loneliness and depressive feelings in different age groups, as there may be developmental differences in which of the two is strongest related to mood levels.

Suggestions for Further Research

In the reflections on the main findings, we have already provided some suggestions for further research. Some of those suggestions will be discussed in further detail in the present section, and some new suggestions for further research will be introduced.

Social Contexts. In the present dissertation, we showed that adolescents experienced the highest levels of state loneliness and negative affect when alone. As was mentioned before, one of the limitations of these analyses was that we did not examine whether adolescents chose to be alone and what adolescents were doing during their time alone. Hence, further research should measure the extent to which adolescents chose to be alone as this likely influences how they experience the time alone. Further, previous
research has indicated that people may differ in the extent to which they perceive solitude as positive or negative in general (e.g., Teppers et al., 2013). These attitudes towards solitude are likely to impact how adolescents perceive and experience situations alone. Hence, further research should examine relations between attitudes towards solitude, the amount of control adolescents experience over the situation (i.e., being alone), and solitude.

Further, as new technologies such as smartphones and tablets are often used by adolescents and social media become increasingly popular, it is likely that adolescents were not really alone, but instead were virtually interacting with their friends and peers, which we did not measure in the present thesis. This would indicate that the physical social environment adolescents are in may not be the only influence on adolescents feelings of loneliness, as the virtual social environment is also likely to play a role. There are several ways through which adolescents can contact their peers virtually. On the one hand, adolescents may use more traditional ways such as calling or email to keep in touch with each other. On the other hand, new developments have made it even easier to maintain contact, through for example mobile instant messaging (e.g., WhatsApp messenger) and the availability of social media (e.g., Facebook, Twitter). There are several reasons why future research should focus on examining the role of online communication in adolescent loneliness. First, the relation between trait loneliness and online communication still remains unclear, as opposite results are found. Some studies found preliminary evidence that lonely adolescents compensate for their lack of (offline) social connection by seeking contact online (e.g., Valkenburg & Peter, 2007), which is in line with the social compensation hypothesis. On the other hand, other research suggested that only non-lonely adolescents use more online communication, as an extension of their offline social network (e.g., Correa, Hinsley, & de Zuniga, 2010; Gosling, Augustine, Vazire, Holtzman, & Gaddis, 2011), which is in line with the rich-get-richer hypothesis. Although the social compensation hypothesis states that trait lonely individuals could use online communication to compensate for their lack of social relations, very few studies have examined whether this is actually the case. Therefore, further research is needed to find out to what extent trait lonely adolescents use online communication and how they use it. A second reason why it is important to examine the role of online communication in loneliness, is that very little is known about the consequences of using online communication. In this line of research, two contrasting hypotheses have been formulated (e.g., Valkenburg & Peter, 2007). The reduction hypothesis states that virtual contact reduces the closeness of adolescents’ friendships, because online friendships are less close than offline friendships, and time spent online reduces time spent with offline friends. On the other hand, the stimulation hypothesis states that virtual contact encourages the closeness of friendships, as adolescents may be more likely to disclose intimate thoughts and feelings online, which could increase the quality of friendships. Support was found for the stimulation hypothesis, but only for adolescents who used internet to communicate with already existing (i.e., ‘offline’) friends (Valkenburg & Peter, 2007). This particular study shows that online communication may have positive effects when it is used to strengthen already existing relationships. However, further research is necessary to disentangle when and how trait lonely adolescents use online communication, and how this online communication affects their future trait loneliness levels. In addition, the studies that have examined online communication up to now have mainly focused on one-time assessments, measuring how adolescents use online communication in general. Very few studies have examined online communication in real-life, by using momentary assessments. This is an important direction for future research, as knowing how adolescents exactly use online communication and how this affects them directly, can provide important starting points for interventions. Further, examining online communication in real-life would also make it possible to examine whether virtual communication can fill the void of lack of company when adolescents are alone.

Only two studies have examined the real-life effects of online communication, by investigating Facebook use (Deters & Meehl, 2012; Kross et al., 2013). Facebook is one of the most often used social media sites in the world, especially in adolescence and young adulthood (Stichting Mijn Kind Online & Kennisnet, 2013). The first study used an experimental design in late adolescents (Deters & Meehl, 2012), and instructed participants in the experimental condition to post more on Facebook than they usually did, whereas the control condition did not receive instructions. Participants in the experimental condition significantly decreased in loneliness, because they felt more connected to their friends. These findings indicate that intensive posting on Facebook may reduce feelings of loneliness. Still, this study did not examine direct effects of Facebook use on loneliness or wellbeing. This was done in the second study (Kross et al., 2013), which used an ESM design to examine direct effects of Facebook use on wellbeing in late adolescents. This study showed that higher levels of Facebook use at a given moment were related to lower levels of wellbeing at the next time-point, which would indicate that Facebook use has a negative direct impact on well-being. Further, the extent to which participants felt lonely at a given moment was related to increased Facebook use, which shows that people used Facebook more when they felt lonely. However, the study did not examine whether Facebook use also caused subsequent feelings of loneliness. The findings from these two studies seem to be contradictory, as one study found that increasing status updates on Facebook decreases loneliness, whereas the second study showed that using Facebook decreased wellbeing immediately. Hence, further research is necessary, as it is likely that the type of activity adolescents engage in on Facebook determines whether the effects are positive or negative. In addition, these studies were conducted in late adolescents, whereas they might use social media differently compared to early adolescents. Future research needs to include different age groups in order to examine possible age differences in the relation between social media use and wellbeing.

In sum, future research needs to combine questionnaire assessments in which trait loneliness and general use of online communication is measured, with Experience...
Sampling research in which the use of online communication in different contexts can be examined, as well as direct antecedents and consequences of online communication. In this way, it is possible to investigate how trait lonely adolescents use virtual media and how this affects their wellbeing.

**Socio-cognitive model.** Research on the two characteristics of the socio-cognitive model is still in its infancy and further research is warranted. As was shortly touched on earlier, there are several things to consider in future research on this topic. First of all, as our results on the subjective experience of these characteristics in daily life were partly in contrast with research that used more objective measures (e.g., Cacioppo et al., 2009), it is important to use these different types of measures in one sample of adolescents. In this way, it would be possible to explore whether adolescents who are found to be hyper- or hyposensitive to objective measures respond similarly to subjective measures in daily life. Secondly, differences in these characteristics between chronic and temporarily lonely individuals need to be explored. Thirdly, we do not know how these characteristics further affect trait loneliness levels. As was hypothesized by Cacioppo and Hawkley (2009), it could be that these characteristics lead to a negative spiral, in that levels of loneliness are increased or maintained, which in turn further contributes to the maintenance of the two characteristics. Further, as was mentioned before, our results show that lonely adolescents respond differently to their environments, which is in line with previous research on the belonging regulatory system, which showed that lonely adolescents are more sensitive to social cues than non-lonely adolescents (Gardner et al., 2005). These findings and the findings in the present thesis indicate that lonely adolescents do not have difficulties with the second step of the belonging regulation model, but we do not know whether and how lonely adolescents use these social cues to restore their relationships (i.e., the behavioral phase of the belonging regulation model). This highlights the importance of using a longitudinal design, in which the responses of lonely adolescents to their heightened environmental sensitivity is examined.

We suggest that further research on the socio-cognitive model combines a longitudinal design with different types of measures for both characteristics, that is, using experimental tasks, fMRI research, and the ESM to measure responses to objective and subjective social stimuli. In this way, it is possible to examine possible differences in the effects of objective, non-personalized stimuli versus subjective, personally relevant social stimuli. Specifically, a possible way to measure hypersensitivity to social threat and hyposensitivity to social reward in an objective, non-personalized way is by using experimental tasks in which attention to threatening and rewarding stimuli is examined. This can be done by means of eye-tracking, in which the attention to socially threatening or positive social pictures or video clips is measured (see for example Qualter, Rotenberg, et al., 2013). Another way to examine attention to positive and negative stimuli is by using a dot-probe task, in which the processing of positive, negative, and neutral stimuli is examined (for an overview of attention bias measures, see Bar-Haim et al., 2007).

Further, we would suggest to use fMRI to measure brain activity in response to objective, positive and negative social stimuli, similarly to the study by Cacioppo et al. (2009) that did find evidence for hyposensitivity to social reward. In addition to this, it may also be interesting to use personally relevant social stimuli, instead of general social stimuli. A possible way to do this may be by using a social imagery task (Frenen et al., 2011). In such a task, scripts of social situations are read to participants while they are in the scanner, and they are instructed to imagine that the situation described in the script was actually happening to them at that moment. These scripts may reflect positive social situations to measure sensitivity to social reward (e.g., receiving a compliment from a close friend) and negative social situations to measure sensitivity to social threat (e.g., noticing that your friends are negatively gossiping about you). The main advantage of such a task is that it enables the person to think about personally relevant situations, but still the task and the scripts are similar across individuals, which is necessary to be able to compare results between participants.

Using a social imagery task makes it possible to examine whether the difference in findings between the fMRI study and the present study is due to the objective nature of the stimuli in the fMRI study, in comparison to the subjective measures used in the present dissertation. Finally, when both trait loneliness and the two characteristics are measured longitudinally, it is possible to examine (1) whether the chronicity of loneliness affects the two characteristics, (2) whether the two characteristics precede loneliness, or develop once someone experiences loneliness, and (3) whether and how adolescents respond to these characteristics in their behavior.

**Part II: Genetic effects**

**Reflections on Main Findings**

**Developmental Perspective.** First of all, our longitudinal analyses showed that trait loneliness was highest in early adolescence, and slowly decreased throughout adolescence. This was in line with previous research that examined the development of loneliness from childhood into adolescence, which showed a peak in early adolescence as well (Qualter, Brown, et al., 2013). These findings highlight the importance of examining loneliness in early adolescence.

In the past, most research examining genetic effects has used one-time assessments to measure the outcome of interest. The studies in the present dissertation showed that taking a developmental perspective by measuring the development of loneliness throughout adolescence has provided insights that we would not have found by using one-time assessments (i.e., relation between 5-HTTLPR and the slope of loneliness in Chapter 7, relation between OXTR and the slope for girls in Chapter 9, and the OXTR x DRD2 interaction on the slope in Chapter 9). The importance of taking a developmental...
perspective is further highlighted by the findings of previous behavioural genetic studies on loneliness, which showed that the heritability estimates of loneliness differ between children (45-55%) (Bartels et al., 2008; McGuire & Clifford, 2008), adolescents (75%) (Waaktaar & Torgersen, 2012), and adults (55%) (Boomsma et al., 2005). This could indicate that the effects of genes differ between development phases, which has also been found in previous studies with different genes on different outcomes (Hilt et al., 2007; Sebastian et al., 2010). These developmental differences may be caused by pubertal development. As adolescents go through puberty, their behaviour may change dramatically, which could result in an instability in phenotypes throughout adolescence. This instability may also affect how genes are related to those phenotypes. Further, hormonal changes due to puberty may affect the impact genes have. For example, oxytocin receptors are partly upregulated by estrogen (e.g., Bale & Dorsa, 1995; M. Feng et al., 2009; Quiñones-Jenab et al., 1995), a sex hormone that is particularly present during pubertal development. Therefore, the effects of the OXTR gene may be dependent on the pubertal stage an adolescent is in. The same holds for the 5-HTTLPR gene, as estrogen is found to affect serotonergic activity, possibly by regulating serotonin receptor numbers (for review, see Rubino, Schmidt, & Roca, 1998). Further, research has indicated that gonadal steroid hormones (e.g., estradiol) affect dopaminergic function in the forebrain (see Kuhn et al., 2010; for a review of gonadal hormone influences on dopaminergic function), which could also influence the effects of the DRD2 gene. As these findings indicate that the biological age (i.e., pubertal stage) of adolescents may influence the effects of certain genotypes, further research should focus on examining the role of pubertal development, in addition to differences in gene effects due to chronological age. In sum, our findings, combined with previous research on pubertal development, indicate that further research in tightly specified (biological) phases is necessary to elucidate the developmental differences in gene effects.

**Differences in Findings.** Previous research has shown that loneliness is moderately heritable, and the genetic studies that are presented in this dissertation add to this by showing how candidate genes play a role in adolescent loneliness. In several chapters, we found significant relations between different genotypes and gene-environment interactions and trait loneliness. These findings are not yet replicated and hence have to be interpreted with some caution. We examined main effects and gene-environment interactions with the same genes in relation to state loneliness, which could provide a more complete picture. Unexpectedly, some of these results on state loneliness differed from the findings on trait loneliness (see Table 1 for an overview of results). For the 5-HTTLPR gene, we found that adolescents carrying a short allele remained stable in loneliness over time, and that they were more susceptible to maternal support (in the longitudinal study). In the ESM study, no direct relation was found between the 5-HTTLPR and state loneliness. Further, we did find that adolescents carrying the short allele or a Lg allele (i.e., less efficient variants of the 5-HTTLPR gene) were not affected by positive company, whereas LaLa genotypes (i.e., the more efficient variant of the 5-HTTLPR gene) benefited from higher levels of positive company. The GxE findings in the longitudinal versus ESM study differ from each other as to which genotypes are more susceptible to their environment. Regarding the DRD2 genotype, it was found that A2A2 genotypes were more susceptible to parental support in the longitudinal study, whereas this gene-environment interaction was not significant for state loneliness (in the ESM study).

In both studies, no direct relations were found between the DRD2 genotype and trait and state loneliness. For the OXTR genotype, we found that girls carrying the GG genotype of the OXTR gene remained stable in trait loneliness over time. On the other hand, we found in the ESM study that girls carrying the GG genotype had lower levels of state loneliness than girls carrying an A allele. In addition, in the ESM study a gene-environment interaction was found, in that adolescents carrying the GG genotype were more negatively affected by negative company. No gene-environment interactions were found in the longitudinal study.

| Table 1 Differences in Genetic Effects Between Trait and State Loneliness |
|--------------------------|-----------------|-----------------|
| **Gene**                | **Trait loneliness** | **State loneliness** |
| 5-HTTLPR                | Direct relation  | S-allele carriers remained stable in loneliness | - |
| GxE interaction         | S-allele carriers were more susceptible to parental support | LaLa genotypes were more positively affected by positive company |
| DRD2                    | Direct relation  | -               | - |
| GxE interaction         | A2A2 genotypes were more susceptible to parental support | - |
| OXTR                    | Direct relation  | GG genotypes remained stable in loneliness | Girls carrying A allele had higher state loneliness |
| GxE interaction         | -               | A-allele carriers were more negatively affected by negative company |

There may be several explanations for this complex pattern of findings. First of all, we should state that contradictory findings are relatively common in genetic studies and previous research has suggested that this may be due to differences between studies in sample composition (e.g., sex, ethnicity, age), research designs (e.g., longitudinal, cross-sectional, ESM, or experimental studies), and operationalizations of the phenotype and the environment (e.g., trait versus state measures, major life events versus minor hassles, etc.).
A possible explanation for differences in findings in the present dissertation may be that we are comparing findings on different phenotypes (i.e., trait loneliness versus state loneliness). It could be that genes have different effects on variables representing different levels of measurement. Therefore, we could try to explain our findings by combining the effects of state and trait loneliness. For example, when we look at the findings with the 
OXTR gene, the results on trait loneliness show that A carriers decrease in trait loneliness over time. The results on state loneliness showed that A carriers responded more negatively to negative company in that they experienced higher levels of state loneliness. These results may seem contradictory, however, it could also be normative to experience increased levels of state loneliness in response to negative company, in that experiencing state loneliness serves as a signal that warns the individual that something is wrong in the social context, and actions have to be undertaken to restore social relations. In this way, A carriers may benefit from experiencing higher levels of state loneliness in response to negative company, but only when this would encourage them to act in response to these feelings and restore their social relationships. As a result, A carriers may decrease in trait loneliness over time, whereas GG genotypes may remain stable in trait loneliness, as they do not respond to negative environments, and may not be encouraged to change these negative situations.

However, explanations of this order are not substantiated by our data, and are therefore purely speculative. These speculative explanations can only be tested in future research by administering longitudinal questionnaire assessments measuring trait loneliness and Experience Sampling data measuring state loneliness, in the same adolescents. When similar differences in findings are present in such a study, it will indicate that it is due to the type of measurement.

### Gene-Environment Interactions

In the present dissertation, we did not find gene-environment interactions on trait loneliness with the OXTR gene (Chapter 9) and on state loneliness with the DRD2 gene (Appendix I). However, we did find evidence for gene-environment interactions on trait loneliness with the S-HTTLPR gene in Chapter 7 and the DRD2 gene in Chapter 8 (i.e., interactions with parental support), on state loneliness with the OXTR gene in Chapter 10, and with the S-HTTLPR gene in Appendix I (i.e., interactions with negative company and positive company, respectively). The gene-environment interactions on trait loneliness were cross-over interactions (e.g., “non-removable interactions”, Belsky & Pluess, 2009), and are indicative of differential susceptibility (i.e., showing that certain genotypes are more susceptible to both positive and negative environments). However, we should note that we did not test whether the slope of the “susceptible” subgroup differed significantly from the slope of the “non-susceptible” subgroup, which means that we cannot conclude that the subgroups significantly differed from each other on both ends of the continuum, that is, for better and worse. By examining and interpreting interactions in terms of differential susceptibility, the focus is not so much on the outcome of interest, but more on which genotype is more affected by the environment, with the only restriction that the environment can be both positive and negative (i.e., for better and worse). This is also shown in several reviews and meta-analyses on differential susceptibility (Bakermans-Kranenburg & van IJzendoorn, 2011; van IJzendoorn et al., 2012), in which all phenotypes are taken together. This could be informative, when it is indeed the case that the phenotype does not matter and it is only an issue of which genotype encounters which environment. Although the findings from these meta-analyses may indicate which genotypes are more susceptible, almost no studies have tried to explain or examine why these genotypes are more susceptible. Hence, we do not know what it is that makes these individuals more susceptible. The underlying mechanisms of differential susceptibility may represent possible endophenotypes, that explain why certain genotypes are more susceptible to their environment. Hence, when it is indeed the case that certain genotypes increase susceptibility to the environment, instead of being directly related to phenotypes, it may make more sense for further research to examine these underlying mechanisms instead of examining gene-environment interactions in relation to different phenotypic outcomes.

### Gene-Environment Correlations

In the present thesis, we examined gene-environment interactions. However, previous research has also provided evidence for a related, though different construct, gene-environment correlations (rGE), which are present when correlations occur between genes and the environment. When gene-environment correlations are found, it indicates that certain genes may predispose an individual to experience certain environments. There are several forms of rGE (Rutter, 2007). Passive rGE are present when the adolescents’ genes, which are inherited from their parents, are related to environmental factors. These rGE come into play because parents shape their children’s rearing environment, based on their genetic make-up, but also pass on their genes to their children. In this way, a correlation between the adolescents’ genotypes and the environment may reflect a correlation between the parents’ genotypes and the environment. In our study for example, finding a correlation between parental support and certain genes would indicate that parental support has a genetic basis. Evocative rGE refer to a situation in which an adolescent evokes certain responses from the environment, based on his/her genetic make-up. Active rGE occur when genetic factors make an adolescent choose or select certain environments. In our studies on trait loneliness, no significant gene-environment correlations were found, which indicates that parental support does not have a genetic basis (passive rGE) and adolescents do not evoke certain levels of parental support based on their genetic make-up (evocative rGE). In the ESM study however, we found evidence for small rGE between perceptions of company and all three genotypes. These findings could indicate that adolescents with certain genotypes actively choose more negative environments (active rGE), or that they evoke certain negative responses from their environment (evocative rGE). (Passive rGE are not applicable here, as the environment did not solely consist of parents.) On the other hand, we operationalized our environment as the subjective perceptions of adolescents, instead of objective
environments. As we found that these positive and negative perceptions are related to both loneliness and depression, these perceptions may reflect a common basis in both phenotypes, which could explain why certain genes are related to both loneliness and depression. Further research should focus on examining relations between candidate genes, perceptions of company, and internalizing problems in order to find out whether the common genetic basis in internalizing problems is due to these perceptions of company.

**Suggestions for Further Research**

**Operationalization of Phenotype.** The phenotype of trait loneliness may require further specification. Transient feelings of trait loneliness may have a different genetic basis than chronic feelings of trait loneliness. As transient feelings of loneliness are expected to have positive consequences because these feelings may serve as a motivational state, specifically these transient feelings of loneliness are expected to be evolutionary relevant. In the present dissertation, we measured the development of loneliness throughout adolescence, but we did not further specify subgroups of lonely adolescents into chronic versus non-chronic lonely adolescents, for example. If research was to continue to examine relations between genes and trait loneliness, it would be interesting to examine whether adolescents who experience loneliness as a motivational state, that is, who respond to loneliness by trying to re-establish their social relationships, have a different genetic make-up than adolescents who do not respond to the experience of loneliness in a positive way. These responses to loneliness are part of the belonging regulatory model of Pickett and Gardner (2005), representing the capacity to perceive and detect social cues and opportunities for social inclusion and the actual behavior or social skills that are needed to respond to those cues in an appropriate manner. These characteristics of lonely individuals should be further examined in relation to genetic factors, as we will discuss in the next section.

Further, previous research has indicated that the questionnaire that is used to measure the phenotype of interest has an impact on whether or not the phenotype is related to specific candidate genes. For example, a meta-analysis on anxiety related personality traits showed that the 5-HTTLPR gene was differentially related to neuroticism (i.e., significant versus non-significant), depending on the questionnaire that was used (Munafo et al., 2005). Hence, future studies examining genetic effects in relation to loneliness could consider including different measures of loneliness, to make sure that the findings are not attributable to the questionnaire that was used. In addition, replication studies should use the same questionnaire as we did, so that non-replication is not due to the instrument.

**Endophenotypes.** Although the size of our genetic effects is similar to effect sizes in other studies (Ioannidis, Trikalinos, & Khoury, 2006), our findings are relatively small and we found that results on trait and state loneliness differ from each other, which raises the question whether future research should continue in this direction. Although we would like to stress that our studies were a first step in examining possible candidate genes in a phenotype (i.e., loneliness) that showed moderate levels of heritability in behavioral genetic studies, we might have to take a step back at this point. Because it has been shown that the genes that we examined play a role in many different phenotypes (i.e., pleiotropy; Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013), it may make more sense to examine what those phenotypes have in common that may explain why they share relations with similar genes. A possible way to do this is by examining endophenotypes, as was described in the Introduction of the present thesis. As the genes in the present dissertation are also related to other types of internalizing problems such as depression (Karg et al., 2011) and anxiety (Schinka, Busch, & Robichaux-Keene, 2004), which are closely related to loneliness, it makes sense to examine endophenotypes for internalizing problems. Possible endophenotypes for internalizing problems could be, for example, hypersensitivity to social threat, one of the characteristics of the socio-cognitive model, as this characteristic has been found to play a role in loneliness (Chap 4 and 5), depression (Davey et al., 2008), and anxiety (Bar-Haim et al., 2007). As we describe in our suggestions for further research on the characteristics of the socio-cognitive model (i.e., Part II), a good way to examine hypersensitivity to social threat would be by combining objective measures of hypersensitivity (e.g., by using experimental tasks) with subjective measures of hypersensitivity in real life. This could provide a clear and interesting endophenotype that includes both objective and subjective measures. As this endophenotype is related to different types of internalizing problems, finding relations between genes and this endophenotype would provide evidence for a possible mechanism that explains why the same genes are related to different phenotypes.

In addition, another possible endophenotype for internalizing problems may lie in social information processing. Previous research has indicated that, compared to low lonely adolescents, lonely adolescents have increased incidental social memory and are better in decoding social cues (Gardner et al., 2005), showing that lonely adolescents have a adequately working social monitoring system, that is, they are able to monitor the environment for social cues that could enhance social inclusion (Pickett & Gardner, 2005). These findings provide support for the evolutionary model of loneliness, in that loneliness is a motivational state that increases sensitivity to environmental cues in order to restore social relations. In addition, social information processing has been related to other often examined phenotypes, such as depressive feelings (e.g., Dozois & Dobson, 2001). Therefore, further research could examine different aspects of social information processing as possible endophenotypes for internalizing problems. There are already many studies present that examined relations between genes and certain aspects of social information processing (e.g., see for meta-analysis on 5-HTTLPR and attention to negative emotional stimuli Pergamin-Hight, Bakermans-Kranenburg, van IJzendoorn, & Bar-Haim, 2012). However, those studies most often use static emotional stimuli in experimental tasks (e.g., dot-probe task). It would be interesting to examine whether enhanced decoding of more
realistic social cues would be related to certain genotypes. For example, by using eye-tracking studies, future research could examine whether adolescents with certain genotypes show increased or decreased visual attention to social cues in video clips (e.g., Klin, Jones, Schultz, Volkmar, & Cohen, 2002).

**Polymorphisms.** In the present dissertation, we used single polymorphisms to represent the different genes, because these polymorphisms were often examined in previous research. However, other polymorphisms in the same genes may also play a role in loneliness. For example, research has shown that next to the rs53576 SNP we used, other SNP’s in the OXTR gene are also related to internalizing problems (e.g., rs2254298; Costa et al., 2009) and rs2268498 (Montag et al., 2011). In addition, research by Neville et al. (2004) has shown that the DRD2 Taq1A SNP is actually not located in the DRD2 gene, but in a neighboring gene, the ankyrin repeat and kinase domain containing 1 (ANKK1), which is often inherited together with the DRD2 gene. Studies on alcohol dependence (Dick et al., 2007) and nicotine dependence (Gelernter et al., 2006) have indeed suggested that the relation between DRD2 and alcohol and nicotine dependence may be attributable to variants of the ANKK1 gene. These findings highlight the importance of including multiple SNPs. In a review on gene-environment interactions, Dick (1999) also suggests that research should not focus on single polymorphisms, but on so-called ‘blocks’ of SNP’s (or haplotypes) that are often inherited together (i.e., high linkage disequilibrium across a group of SNP’s). Because some genes may consist of several blocks of SNP’s, choosing SNP’s from different blocks within the same gene may result in different conclusions. Future genetic research should consider examining these blocks of SNP’s, instead of single polymorphisms.

**Epigenetics.** Although the human DNA sequence is stable and cannot be changed by outside influences, exciting recent developments in molecular genetics have shown that gene expression can be changed by environmental influences (i.e., epigenetics). Hence, although the DNA sequence remains intact, the environment can influence which genes are ‘turned on’ and which genes are ‘turned off’. Simplified, there are two main ways in which the environment can impact on gene expression (e.g. see Curley, Jensen, Mashoond, & Champagne, 2011; J. Feng, Fouse, & Fan, 2007). First, the environment can affect how the DNA is ‘wrapped’ in so-called histones. This wrapping around histones determines which genes are expressed, as only the genes that are not wrapped around a histone can be read and consequently, come to expression. Second, the environment can impact methylation of the DNA. The methylation of DNA determines at which sites of the DNA transcription factors can bind, and hence also determines which genes are expressed, and which are not. Even though research in epigenetics is still in its infancy, there are some studies that indicate that the social environment can affect gene expression (Cole, 2013). For example, a study in older adults showed that individuals who experienced loneliness had up-regulated pro-inflammatory genes and down-regulated antiviral and antibody related genes (Cole et al., 2007). These changes in gene-expression due to loneliness may explain why loneliness is related to detrimental health outcomes (Cacioppo & Hawkley, 2003). These findings show that the experience of loneliness can affect gene expression. Hence, it may not be sufficient to examine only polymorphisms in the genome, because the epigenetic genome seems to play a role as well. Therefore, further research should take epigenetic mechanisms into account.

**Limitations**

In general, we used innovative paradigms to examine our research questions in the present dissertation. By using these methods, we were able to explore exciting new areas in loneliness in adolescence. However, these methods have some limitations as well, that have to be addressed.

**Experience Sampling Method**

There are some issues with the Experience Sampling Method in particular that have to be acknowledged. First of all, the data collection was unsupervised, making it difficult to check whether adolescents filled out the questionnaires themselves, and whether they did it seriously. We did screen the data for strange or unexpected answers, in order to filter out the assessments that were not filled out seriously. Hence the assessments that were not filled out seriously were excluded from our analyses. In addition, it is impossible to conduct ESM studies with supervision, as the main advantage of this design is that adolescents fill out the questionnaires in real life and at unexpected moments.

Further, in all samples, the assessments occurred quasi-randomly (i.e., at random times, but between predefined time points). The most important advantage of random time points is that adolescents were not able to predict when the assessments would occur, and hence could not anticipate on an upcoming assessment (e.g., by changing their daily activities so that it fits with the assessments times). In addition, in the early adolescent sample, adolescents from the same classes participated in the same week. When we would have had similar time-points for all participants, adolescents may have been tempted to discuss the questions with each other, or fill out the questionnaires together. By making the timing of assessments random between persons as well, we tried to prevent this. However, there also are some disadvantages of randomly timed assessments. The main disadvantage of random time points comes into play when examining lagged effects. As the time between assessments differed, the relation between, for example, social context at T1 and state loneliness at T2 may differ between assessments as the time elapsed between the two measures differed. However, in the one study in which we examined lagged effects between two consecutive assessments (i.e., Chapter 2), controlling for the time between assessments did not affect the overall results, showing that at least in those analyses, the random timing did not affect the results.
Further, compliance with data collection in the two Dutch adolescent samples was moderate (i.e., early adolescents filled out 69% of the assessments on average, late adolescents 65%), compared to other studies (e.g., Schneiders et al., 2007) and the compliance in the late adolescent sample from the US (i.e., 80%). There may be several explanations for these relatively lower compliance levels. First, even though the use of smartphones makes the administration of questionnaires easier and less time-consuming, adolescents may not always carry the smartphone with them (e.g., forget to take it with them or carry it in their bag) and hence sometimes miss the buzzing signal. In other paper-and-pencil studies (and in the US data we used), researchers usually use wristwatches that emit a beeping signal when an assessment has to be filled out. Carrying a wristwatch may be easier than carrying a smartphone, which is bigger and more difficult to carry in a pocket. Further, in consultation with the participating schools, we decided to use buzzing signals instead of beeps, as this was less disturbing during school hours. Hence, the type of signaling device (wristwatch versus smartphone) and the type of signal (buzzing signal versus beeps) may have made it more likely that adolescents missed assessments in our samples.

Second, our questionnaires may have been relatively long, with a maximum of 43 questions in the early adolescent sample, and 56 questions in the late adolescent sample. Although adolescents reported that filling out the assessments took around 3-4 minutes, for early adolescents, the length of the questionnaire may have influenced their compliance, as their self-regulatory skills and concentration span may not be as developed as in late adolescents (e.g., Steinberg, 2005). For the late adolescent Dutch sample, the length of the questionnaire was similar to the questionnaires in the US sample, which comprised 64 items. Hence, the length of the questionnaire may not explain the difference in compliance between the late adolescent samples.

Another issue that has to be addressed refers to our measure of hypersensitivity to social threat. It may be questionable whether it is even possible to measure this characteristic in the daily lives of adolescents. Not only do the measures we used in the ESM studies reflect subjective experiences (i.e., perceptions of threat), it may also be questionable whether it really is possible to measure threat in daily life. Is it likely that adolescents experience extreme levels of threat on a 6-day basis? Because of these queries, we further examined within person extreme levels of negative company in early adolescents (Chapter 6). Although these within-person extremes (i.e., peaks) may still reflect relatively low scores on negative company when the range is taken into account, these peaks do indicate that for that specific person, high levels of negative company are experienced. The results from this study showed that, on average, adolescents experienced a number of 3.6 peaks in negative company (range between 1 and 13) during the 6-day sampling period and only 9% of the total sample never experienced a peak. These findings indicate that there is variance in the experience of threat in daily life, and that adolescents do experience within-person extreme levels of threatening and judging company during a 6-day sampling period.

State Loneliness Measure

Only a few studies have measured state levels of loneliness. These studies often used single-item measures (Larson, 1981), or combined a loneliness item with a negative affect item (e.g., ‘sad’; Doane & Adam, 2010). We argued that it would be better to use several items, as this would result in a more reliable measure. We aimed to use items that were easy to fill out. The measure we used was based on four items: ‘I feel lonely, abandoned, isolated and leftout’, that were derived from items used in trait questionnaires (e.g., “how often do you feel isolated from others”; “how often do you feel left out” from the UCLA scale; “I feel abandoned by my friends” from the LLCA scale). From a theoretical point of view, these items reflect certain aspects of loneliness. This is also substantiated by the internal consistency of the scale, which showed that we measured state loneliness reliably. However, one of the limitations of the present dissertation is that we did not further examine the psychometric properties of the state loneliness measure. Further research is necessary to show whether this measure proves to be valid.

Trait Loneliness Measure

One of the limitations regarding the trait loneliness measure in the ESM datasets (i.e., Part I of this dissertation) is that we did not measure chronic levels of trait loneliness, as we only had one assessment. Therefore, we could not differentiate between chronic and temporarily lonely individuals in our samples. As previous research has indicated that chronic lonely adolescents have the highest levels of depressive symptoms and stress, and the lowest levels of self-esteem, compared to temporarily lonely adolescents (Vanhalst, Goossens, et al., 2012), chronic lonely individuals may also experience their daily lives differently. Hence, further research is necessary to investigate possible differences in outcomes between chronic and transient lonely adolescents.

Power Issues

As was mentioned in our general introduction, low power is an issue in most genetic studies (e.g., Ioannidis, Trikalinos, Ntzani, & Contopoulos-Ioannidis, 2003; Munafö et al., 2009). We tried to overcome this power problem by using the ESM, in which we had more power due to the multiple measurements (Finn et al., 2012). However, in the longitudinal study we did not have as many measurements, and hence, we may have been more likely to obtain false positive findings, due to Type I error. On the other hand, this power issue may have been relevant for genetic main effects only, as we have conducted a power analysis in Chapter 9 that showed that we did have enough power to detect small gene-environment interactions in the longitudinal study. Therefore, we are confident that our gene-environment interactions were not due to Type I errors. Still, power remains an issue until our findings are replicated in other studies.
Suggestions for Intervention

Part I: Daily Life Processes

In general, the findings in the present dissertation show (a) that lonely adolescents are more affected by their positive and negative perceptions of company, and (b) that lonely adolescents respond more negatively to being alone but also benefit more from being with intimate company, compared to non-lonely adolescents. However, as was mentioned before, we do not know whether adolescents are aware of these characteristics and use this knowledge to decrease their loneliness levels. A possible way to make adolescents aware of these characteristics is by using personalized Experience Sampling Interventions (Heron & Smyth, 2010, a promising new direction in Experience Sampling research. Individual Experience Sampling data could provide further insight in when, with whom, and during which activities lonely adolescents experience state loneliness, positive affect, and negative affect. This information can be used to provide personalized feedback to adolescents on how they respond to certain situations. In this way, adolescents may become more aware of which situations have negative and positive effects on them, and could use this information to change these situations (e.g., try to increase positive situations).

Of course, it may not be enough to simply advise adolescents to avoid negative situations, when it could be merely their perception or cognitions that are more negative, and not necessarily their environment. As was shown in a meta-analysis on interventions for loneliness (Masi et al., 2011), addressing maladaptive social cognitions was the most effective intervention to reduce loneliness (i.e., Cognitive Behavioral Therapy; CBT), compared to interventions that focused on improving social skills, enhancing social support, and increasing opportunities for social contact. Although this meta-analysis is mainly based on studies examining older adults and trait loneliness, this finding may highlight the importance of changing lonely adolescents cognitions and perceptions as well. Hence, it would make sense to combine personalized feedback on daily characteristics with aspects of cognitive behavioral therapy, so that adolescents become aware of the effects of social contexts, but are also challenged to change their maladaptive cognitions.

To our knowledge, there are no interventions targeting maladaptive cognitions in lonely adolescents specifically. However, it may also be useful to implement more general prevention programs that target maladaptive cognitions. An example of such a program is ‘On Full Power’, a depression prevention program that incorporates CBT to address negative cognitions in adolescents, which has been found to decrease depressive symptoms in an at-risk sample of adolescent girls with elevated depressive symptoms (de Winter et al., 2005).

Part II: Genetic influences

In the second part of this dissertation, we showed that adolescents with certain genotypes were at risk for experiencing both trait and state levels of loneliness. In addition, our results indicated that some genotypes may be more susceptible to environmental effects. Although we cannot intervene in human DNA and change the genetic make-up of individuals, these findings may still provide starting points for prevention and intervention efforts. However, there are several reasons why we will not yet draw conclusions based on the findings in this dissertation. First of all, our studies are among the first to examine genetic effects in trait and state loneliness, and our results have not been replicated. Replication of our results is necessary (Ioannidis, Ntzani, Trikalinos, & Constantinou, 2001), both in normative as well as clinical samples, before any firm conclusions can be drawn. Second, as was mentioned before in this Discussion, we think that genetic research mainly has to focus on examining endophenotypes, in order to obtain a more complete picture of the relations between genes and behavioral phenotypes. When results are more clear, we can think about the implications for clinical practice. Third, multiple genes are found to be related to loneliness, but these main effects are small. Hence, it may not make sense to select participants for prevention or intervention based on their genetic make-up, as the genes only explain a small portion of the phenotype. Fourth, there are some ethical considerations in using genetic information in interventions. It is still unclear whether providing information about certain genetic risks will lead to stigmatization (Springer, Olsson, & Hall, 2008). As is often discussed now regarding individuals that engage in negative health behaviors, knowing which people are at risk for certain disorders could lead to increased insurance premium, or even exclusion from certain health insurances. Hence, if and when genetic information will be used for intervention purposes, clear guidelines are necessary to prevent stigmatization.

However, despite these reasons not to use genetics in prevention and intervention efforts, gene-environment interaction research may provide an interesting direction for clinical practice. Although further research is still necessary on this topic as well, gene-environment interactions often explain a larger proportion of phenotypic variance compared to genetic main effects. Further, although we cannot change the DNA sequence, we often can change the environment. Therefore, when we know that individuals with specific genotypes are more susceptible to the environment, or respond more negatively to certain environmental stressors, we can attempt to change these environments for these individuals specifically. As we showed in the present dissertation that individuals with certain genotypes are more affected by the perceptions they have of others (i.e., negative company in ESM study, parental support in longitudinal study), these perceptions may provide a good starting point for interventions. In addition, research has recently shown that individuals with certain genotypes (i.e., 5-HTTLPR short allele carriers) benefited most from Attention Bias Modification training (i.e., ABM, E. Fox, Zougkou, Ridgwell, & Garner, 2011), which might indicate that short allele carriers are more responsive to therapeutical
Interventions than long-long genotypes. This line of research could provide important directions for further research, as the knowledge on which genotypes are responsive to which types of interventions can result in personalized therapy.

Concluding Remarks

The findings in the present dissertation provide important new insights in the daily experiences of lonely adolescents as well as the genetic underpinnings of both trait and state loneliness. By using an exciting new methodology (i.e., ESM), we have shown that high lonely adolescents respond differently to social contexts compared to low lonely adolescents, in both negative and positive ways. These findings concur with the evolutionary model of loneliness (Cacioppo, Hawkley, et al., 2006), but partly contradict research on the socio-cognitive model of loneliness (Cacioppo & Hawkley, 2009). Further, the genetic studies in the present thesis provide some evidence for candidate genes that may play a role in trait and state levels of loneliness, but replication of these findings is necessary before any firm conclusions can be drawn. Further research on endophenotypes is needed to elucidate overlap in genetic findings between different phenotypes. When replicated, the findings in the present dissertation may have important implications for interventions on loneliness, as the daily experiences of lonely adolescents could represent a direct starting point for intervention purposes. However, future research should focus on investigating whether these daily characteristics maintain or diminish feelings of loneliness.
References


Dutch Summary (Nederlandse Samenvatting)

Eenzaamheid is een onaangenaam gevoel dat ontstaat als een reactie op het ervaren van een verschil tussen het gewenste en daadwerkelijke sociale netwerk van een persoon. Tijdelijke gevoelens van eenzaamheid worden door iedereen weleens ervaren. Tijdelijke eenzaamheid heeft niet per se negatieve gevolgen, omdat deze gevoelens mogelijk mensen motiveren om sociaal contact te zoeken zodat ze deze negatieve gevoelens kunnen verminderen. Langdurige gevoelens van eenzaamheid kunnen daarentegen ernstige gevolgen hebben voor de mentale en fysieke gezondheid, zoals angst, depressie, verhoogd risico op hart- en vaatziekten en verminderde slaapkwaliteit. Onderzoek heeft ook aangetoond dat eenzaamheid de kans op vroegtijdig overlijden met 50% vergroot. Deze negatieve gevolgen benadrukken het belang om te onderzoeken welke factoren eenzaamheid veroorzaken en in stand houden. Vanwege het toenemende belang van sociale relaties in de adolescentie en belangrijke sociale transities in deze periode (e.g., overgang naar middelbare school voor jongere adolescenten en gaan studeren voor oudere adolescenten), focussen we op het onderzoeken van eenzaamheid in de adolescentie.

Deel I: Processen in het Dagelijks Leven

De meeste studies onderzoeken eenzaamheid als een relatief stabiele karaktertrek (trait), door middel van vragenlijsten. Eenzaamheid hoeft echter geen stabiele karaktertrek te zijn, maar kan fluctueren onder invloed van bijvoorbeeld de sociale context waarin men zich bevindt. Er is zeer weinig bekend over deze momentane gevoelens van eenzaamheid in het dagelijks leven. In het eerste deel van dit proefschrift hebben we momentane eenzaamheid onderzocht bij jongere en oudere adolescenten.

Daarnaast is er weinig bekend over hoe eenzame jongeren hun dagelijks leven ervaren. Volgens een socio-cognitief model worden eenzame mensen gekarakteriseerd door twee kenmerken: overgevoeligheid voor sociale dreiging en ondergevoeligheid voor sociale beloning. Deze twee kenmerken kunnen eenzaamheid veroorzaken en in stand houden. In het huidige proefschrift hebben we deze twee kenmerken onderzocht in het dagelijks leven van jongere en oudere adolescenten. We hebben hierbij gebruik gemaakt van de Experience Sampling Methode (ESM) waarbij we meerdere keren per dag, een aantal dagen lang jongeren vroegen om een korte vragenlijst in te vullen. Een belangrijk voordeel van deze methode is dat de ecologische validiteit hoog is, omdat jongeren rapporteren over hun gevoelens in hun natuurlijke omgeving. Daarnaast
rapporteren ze deze gevoelens op het moment dat ze dit ervaren, waardoor de kans op een herinnerings-bias (recall bias) verkleind wordt.

**Hoofdstuk 2**

In dit hoofdstuk hebben we onderzocht in welk type sociale omgeving jonge adolescenten (13-15 jaar) momentane eenzaamheid ervaren. Uit de bevindingen bleek dat jongeren het meest eenzaam waren als ze alleen waren, in vergelijking met situaties waarin ze in gezelschap waren. Wanneer jongeren in gezelschap waren, ervaarden ze de hoogste eenzaamheid op school en met klasgenoten en de laagste eenzaamheid thuis en op andere locaties, met familie en vrienden. Zowel jongens als meisjes waren minder eenzaam bij vrienden als ze het moment daarvoor alleen waren dan als ze het vorige moment ook al bij vrienden waren. Dit kan erop duiden dat jongeren opgelucht waren wanneer ze met anderen waren en zich daardoor minder eenzaam voelden. Daarnaast lieten de jongeren een ‘spill-over’ effect zien van alleen zijn als zij in het gezelschap van familie kwamen na een periode alleen te zijn geweest. Dit betekent dat jongeren nog steeds eenzaamheid ervaarden doordat ze op een vorig moment alleen waren, maar al waren ze inmiddels in het gezelschap van familie. Deze bevindingen duiden erop dat vrienden en niet familie, een buffer kunnen vormen voor de negatieve gevolgen van alleen zijn.

**Hoofdstuk 3**

In Hoofdstuk 3 hebben we de relatie tussen trait eenzaamheid (eenzaamheid gemeten als een karaktertrek) en state eenzaamheid (momentane gevoelens van eenzaamheid) onderzocht in drie verschillende onderzoeksgroepen: jonge adolescenten uit Nederland (14 jaar), oudere adolescenten uit Nederland (19 jaar) en oudere adolescenten uit Amerika (19 jaar). We hebben getoetst of de differential reactivity hypothese opgaat, die stelt dat eenzame mensen meer stress ervaarden omdat ze, in vergelijking met laag eenzame mensen, negatiever reageren op bepaalde gebeurtenissen. We vonden bewijs voor deze hypothese in alle groepen: hoog eenzame jongeren reageerden negatiever op alleen zijn dan laag eenzame jongeren, omdat ze hogere momentane eenzaamheid ervaarden in die situaties. Anderzijds profiteerden hoog eenzame jongeren ook meer van gezelschap dan laag eenzame jongeren (met name intimi gezelschap), omdat hun momentane eenzaamheid sterker daalde op momenten dat zij met anderen waren. Deze bevinding bevestigt de differential reactivity hypothese: eenzame jongeren reageren anders op zowel negatieve als positieve omgevingen. Een verschil werd gevonden tussen jongere en oudere adolescenten. Bij hoog eenzame jongere adolescenten zorgde de aanwezigheid van niet-intimi gezelschap zoals klasgenoten niet voor een verlaging van momentante eenzaamheid, terwijl dit bij hoog eenzame oudere adolescenten wel het geval was.

**Hoofdstuk 4 en 5**

Hoofdstuk 4 en 5 beschrijven twee Experience Sampling studies, waarin onderzocht is of de twee kenmerken van het socio-cognitieve model (overgevoeligheid voor sociale dreiging, ondergevoeligheid voor sociale beloning) terug te vinden waren in een jongere en oudere adolescente groep. De bevindingen lieten zien dat in beide groepen hoog eenzame jongeren, in vergelijking met laag eenzame jongeren, meer negatieve en minder positieve percepties hadden van hun gezelschap. We vonden bewijs voor overgevoeligheid voor sociale dreiging: hoog eenzame jongeren lieten sterkere negatieve reacties zien op negatief gezelschap dan laag eenzame jongeren; zij hadden een grotere stijging in negatieve gevoelens en een grotere daling in positieve gevoelens als zij in negatief gezelschap waren. De bevindingen voor ondergevoeligheid voor sociale beloning waren in tegenstelling met het socio-cognitieve model; we vonden dat hoog eenzame jongeren juist sterkere (en niet verminderde) positieve reacties hadden op positief gezelschap dan laag eenzame jongeren (i.e. een grotere daling in negatieve gevoelens in positief gezelschap).

**Hoofdstuk 6**

In hoofdstuk 6 hebben we in jonge adolescenten onderzocht of en wanneer zij relatief extreme negatieve sociale situaties ervaren (‘pieken in negatief gezelschap’). Uit de resultaten bleek dat jongeren het vaakst een piek in negatief gezelschap ervaren als zij met klasgenoten waren (in vergelijking met familie, vrienden, en anderen), op weekdagen (in vergelijking met weekenddagen), en in de ochtend (in vergelijking met de middag en avond). Verder vonden we dat sommige jongeren nooit een piek ervaarden in negatief gezelschap (dit waren met name jongens) en dat sommige jongeren geen stijging lieten zien in negatieve gevoelens in reactie op een piek (dit waren met name jongens en jongeren laag in eenzaamheid). Tot slot lieten de resultaten zien dat eenzame jongeren hogere pieken hadden in negatief gezelschap, maar niet meer pieken, en dat zij negatiever reageerden op pieken dan laag eenzame jongeren; hoog eenzamen ervaarden meer negatieve gevoelens gedurende piekmomenten.

**Deel II: Genetische Invloeden**

De evolutionaire theorie van eenzaamheid beargumenteert dat het ervaren van sociale pijn (eenzaamheid) functioneel en adaptief is. Wanneer een persoon sociale pijn ervaart kan dit het signaal geven om te ontsnappen en sociale relaties te initiëren of herstellen. Vanuit een evolutionair oogpunt hadden mensen die sociale pijn ervaarden in reactie op sociale geïsoleerdheid een grotere kans om te overleven en hun genen door te geven dan mensen die geen sociale pijn ervaarden. De kans op overleving is namelijk groter in een sociale gemeenschap waar voedsel gedeeld wordt en mensen beschermd worden tegen dreiging van buiten. Het ervaren van eenzaamheid en de samengestelde motivatie om het
sociale netwerk te herstellen kan dus de kans op overleven vergroten. Hierdoor kan de erfelijke aanleg die eenzaamheid veroorzaakt doorgegeven worden aan de volgende generatie. Eerder onderzoek heeft al laten zien dat eenzaamheid voor ongeveer 50% erfelijk is. Er zijn echter nog weinig studies bekend waarin onderzocht is welke genen hier een rol bij spelen. In het tweede deel van het proefschrift zijn verschillende kandidaatgenen in relatie tot eenzaamheid in de adolescentie onderzocht.

Hoofdstuk 7

Dit hoofdstuk bestaat uit een longitudinale studie met vijf jaarlijkse metingen, waarin onder 306 jongeren de aanvang en ontwikkeling van eenzaamheid in de adolescentie is onderzocht. Daarnaast hebben we bekeken of een genetische variant in het serotonin transporter systeem (5-HTTLPR) gerelateerd was aan eenzaamheid. Tot slot hebben we onderzocht of deze genetische variant mogelijk in samenspel met ouderlijke steun gerelateerd was aan eenzaamheid. De bevindingen lieten alle eerst zien dat eenzaamheid het hoogst was in de vroege adolescens en daarna langzaam daalde. Dragers van de risicovariant van het 5-HTTLPR gen bleven stabiel in hun eenzaamheidsniveau gedurende de adolescentie, terwijl dragers van het lang-lang genotype daalden in eenzaamheid. Daarnaast vonden we dat dragers van de risicovariant (kort allel) gevoeliger waren voor moederlijke steun: hun eenzaamheid was het hoogst als ze weinig steun ervdden en het laagst als ze veel steun ervaarden. Voor steun van vader werden geen gen-omgeving interacties gevonden.

Hoofdstuk 8

In dezelfde onderzoeksgroep als in Hoofdstuk 7 werd onderzocht of een variant in het dopamine receptor systeem (DRD2 gen) mogelijk gerelateerd was aan eenzaamheid. Uit de resultaten bleek dat er geen directe relatie was tussen het DRD2 gen en de aanvang en ontwikkeling van eenzaamheid. We vonden echter wel een samenhang tussen dit gen met ouderlijke steun: dragers van de A2A2 variant (niet risicovariant) waren meer gevoelig voor ouderlijke steun, terwijl A1 dragers (risicovariant) niet beïnvloed werden door steun van ouders.

Hoofdstuk 9

In dit hoofdstuk hebben we onderzocht of een genetische variant in het oxytocine receptor systeem (OXTR) gerelateerd was aan eenzaamheid, zowel direct als in samenspel met ouderlijke steun en met de eerder onderzochte varianten, 5-HTTLPR en DRD2. We vonden dat mensen met de niet-risico variant (GG genotype) stabiel bleven in eenzaamheid gedurende de adolescentie, terwijl mensen met de risico-variant (A-alleel) daalden in eenzaamheid. We vonden geen interacties tussen het OXTR gen en ouderlijke steun. Wel was er bewijs voor een gen-gen interactie: jongeren met de risicovariant van het DRD2 gen (A1 alleel) en de niet risicovariant van het OXTR gen (GG genotype) bleven stabiel in eenzaamheid gedurende de adolescentie, terwijl dragers van andere varianten daalden in eenzaamheid.

Hoofdstuk 10

In het laatste hoofdstuk van dit proefschrift onderzochten we of het OXTR gen ook gerelateerd was aan momentane gevoelens van eenzaamheid in het dagelijkse leven van jonge adolescenten. We vonden dat meisjes die de risicovariant droegen van het OXTR gen (A-alleel) hogere momentane eenzaamheid ervonden dan meisjes die de andere variant hadden (GG-genotype). Daarnaast vonden we dat dit gen in samenspel met de omgeving momentane eenzaamheid voorspelde: jongeren met de risicovariant werden meer negatief beïnvloed door negatieve percepties van gezelschap in het weekend, dan jongeren zonder risicovariant.

Conclusie

De bevindingen in dit proefschrift geven nieuwe inzichten in de dagelijkse ervaringen van eenzame adolescenten en de genetische basis van zowel trait als state eenzaamheid. Door gebruik te maken van een nieuwe methodiek (ESM) hebben we laten zien dat hoog eenzame jongeren anders reageren op sociale contexten dan laag eenzame jongeren, op zowel positieve als negatieve manieren. Enerzijds reageren eenzame jongeren negatiever op negatief gezelschap (zoals klasgenoten), anderzijds reageren zij ook positiever op positief gezelschap (zoals vrienden en familie) dan niet eenzame jongeren. Deze bevindingen komen overeen met de evolutionaire theorie van eenzaamheid, maar zijn deels in tegenstelling met het socio-cognitieve model. De genetische studies in dit proefschrift leveren deels bewijs voor een aantal kandidaatgenen die mogelijk gerelateerd zijn aan trait en state eenzaamheid, maar replicatie van deze bevindingen is nodig voordat we conclusies kunnen trekken. Om de overlap in bevindingen tussen verschillende uitkomstmaten (fenotypes) te kunnen verklaren is verder onderzoek naar mogelijke endofenotypes (factoren die een schakel vormen tussen de genen en de uiteindelijke fenotypes) noodzakelijk. De bevindingen uit dit proefschrift kunnen, indien ze gerepliceerd worden, belangrijke implicaties hebben voor interventies op eenzaamheid. De dagelijkse ervaringen van eenzame jongeren in hun sociale omgeving kunnen namelijk een direct startpunt vormen voor deze interventies. Voordat passende interventies opgesteld kunnen worden is het echter van belang te onderzoeken welke dagelijkse ervaringen van eenzame jongeren hun eenzaamheid in stand houden of juist verminderen.
Publications

This Dissertation:
Van Roekel, E., Ha, T., Verhagen, M., Kuntsche, E., Scholte, R. H. J., & Engels, R. C. M. E. (submitted). The negative company we keep: High levels of negative social experiences in early adolescents’ daily lives.

Other publications:
Acknowledgements (Dankwoord)

Dat was het dan! Het zit er (eindelijk) op, dus is het tijd om de mensen te bedanken die me zoveel geholpen hebben.

Allereerst wil ik graag de deelnemende scholen en jongeren bedanken. Zonder jullie was er niets te schrijven geweest! Ik ben me erg bewust dat ik veel van jullie heb gevraagd, ik ben jullie daar zeer dankbaar voor. Ook alle scriptie-studenten die geholpen hebben bij de data-verzameling (en aanrijdingen met vrachtwagens met mij hebben overleefd), heel erg bedankt!

Rutger, dankzij jou heb ik dit project goed kunnen afronden. Door jouw scherpe blik, hands-on-mentaliteit en enthousiasme was ik na afspraken altijd gemotiveerd en enthousiast om weer aan de slag te gaan. Ik heb veel respect voor je kennis, inzicht en ijzersterke geheugen (vaak wist je beter wat er in m'n papers stond dan ik zelf 😊). Bedankt voor je steun en vertrouwen!

Ron, zonder jou was ik niet eens begonnen aan dit proefschrift! Ik ben je erg dankbaar dat je me 5 jaar geleden de kans hebt gegeven om aan dit project te gaan werken. Toen je me vroeg voor dit project had ik geen idee wat polymorfismen waren en leefde ik niet direct warm voor het onderwerp eenzaamheid, maar toch heb je me weten te enthousiasmeren (gelukkig)! Ik vond het erg fijn dat je deur altijd openstond en je altijd tijd maakte voor me, hoe druk je ook was. Ik heb veel geleerd van onze discussies (ook samen met Maaike), waarbij je vaak ervoor zorgde dat ik zelf tot een oplossing kwam, door de juiste kritische vragen te stellen. Bedankt voor de afgelopen jaren!

Maaike, wat had ik zonder jou gemoeten? Je was toch echt wel een beetje m'n rots in de branding de afgelopen jaren. Je maakte altijd tijd voor me, voor discussies over werkgerelateerde issues, maar ook als me op persoonlijk vlak iets dwars zat. Je nuchtere en relativerende blik heeft me regelmatig verder geholpen. Dank voor je interesse en complimenten, maar ook voor je kritische feedback op m'n stukken.

Luc, vanuit het verre Zuiden heb je mede geholpen om dit project mogelijk te maken. Bedankt voor je vertrouwen in mij, ondanks dat je me niet echt kende in het begin. Je bent eigenlijk een soort wandelende encyclopedie, in de discussies die weadden kon jij altijd zo een paar referenties of onderzoeken opnoemen om je verhaal te ondersteunen. Bedankt ook voor je gastvrijheid tijdens de periode dat ik in Leuven heb mogen werken.

Emmanuel, thanks for your contribution to the peak papers. Your critical view kept us alert and you helped in finding the right analyses for our complicated research questions. I will always remember to avoid too many ingredients in the soup!
John and Stephanie, thank you for your hospitality in Chicago and giving me the opportunity to work on your ESM data. It was a great experience for me, and I learned a lot from you! Hopefully we can continue collaborating in the future.

Thao, what was the key and inspiring about meeting you and starting an ESM study on them. It was fun and inspiring to work together on an ESM study. Thank you for all your help and support over the years. And to all colleagues, we have a good foundation for the years ahead.

Elke en Anne, zusammen mit Hanneke begannen wir an diesem Abenteuer, und entschieden, dass wir allemal durchsichtiger waren und alles so sauber als möglich waren. Elke, sind your vertretung ist unsere Produktivität verdoppelt. Aber noch steckst ist your output of the afgelopen jaar toch een stuk indrukwekkender dan die van ons (twee fijne kindjes ipv één boekje dat niemand meer leest 😊). Dank jijzij zijn we nu elk jaar in Oeteldonk om te vinden, hopelijk kunnen we dat nog lang vervolgen. Anne, in onze tijd samen op een kamer op de 6e hebben we een aardige basis gelegd voor de jaren erna (al is het alleen al in kilo's van al jouw koekjes en snoepjes...). Je bent de meest geïnteresseerde, betrokken en altruïstische persoon die ik ken. Bedankt voor alle gezellige avondjes, stapavondjes en borrels, hopelijk zullen er nog vele volgen!

De Onesiforus crew mag natuurlijk ook niet ontbreken hier. Wim, Marleen, Frank, Inge en Jeroen, het is zo fijn om een groep vrienden te hebben met wie alles altijd vanzelfsprekend en goed is. Wimworm, het was fijn om na de middelbare school ook de studententijd en nu het promoveren met je door te maken. Dank voor alle fijne eet-dates, Dollars avonden en filmverhalen, je bent een toffe vriend. Tjelede en Frank, ook al zien we elkaar niet vaak, we verzanden vanzelf in oude patronen met zinloze discussies over van alles en nog wat. Inge, je bent een fijne vriendin, en het is erg leuk om ook alle PhD's te kennen met je te kunnen delen. Marleen, ik moet nog regelmatig terugdenken aan onze filmnachten inclusief 10 things I hate about you. Riverdance tapdansers en zelfgemaakte tompouchen 😊 We hebben veel lief en leed gedeeld, ik ben onwijs blij met zo'n lieve en sterke vriendin als jij!

Ben & Stien (en Wim), bedankt voor alle avondjes, BBQ's, concerten en Lowlands avonturen. Jullie hebben het leven buiten de PhD een stuk aangenaamer gemaakt. We hebben veel lief en leed gedeeld, ik ben onwijs blij met zo'n lieve en sterke vriendin als jij!

Elke en Anne, samen met Hanneke begonnen we aan dit avontuur, en ondanks dat we allemaal ergens anders terechtkwamen ben ik heel blij dat we elkaar nog steeds zo vaak zien! Elke, sinds jouw vertrek is onze productiviteit verdubbeld. Maar nog steeds is jouw output van de afgelopen jaar toch een stuk indrukwekkender dan die van ons (twee fijne kindjes ipv één boekje dat niemand meer leest 😊). Dank jijzij zijn we nu elk jaar in Oeteldonk om te vinden, hopelijk kunnen we dat nog lang vervolgen. Anne, in onze tijd samen op een kamer op de 6e hebben we een aardige basis gelegd voor de jaren erna (al is het alleen al in kilo's van al jouw koekjes en snoepjes...). Je bent de meest geïnteresseerde, betrokken en altruïstische persoon die ik ken. Bedankt voor alle gezellige avondjes, stapavondjes en borrels, hopelijk zullen er nog vele volgen!

The Desires for crew mag natuurlijk ook niet ontbreken hier. Wim, Marleen, Frank, Inge en Jeroen, het is zo fijn om een groep vrienden te hebben met wie alles altijd vanzelfsprekend en goed is. Wimworm, het was fijn om na de middelbare school ook de studententijd en nu het promoveren met je door te maken. Dank voor alle fijne eet-dates, Dollars avonden en filmverhalen, je bent een toffe vriend. Tjelede en Frank, ook al zien we elkaar niet vaak, we verzanden vanzelf in oude patronen met zinloze discussies over van alles en nog wat. Inge, je bent een fijne vriendin, en het is erg leuk om ook alle PhD's te kennen met je te kunnen delen. Marleen, ik moet nog regelmatig terugdenken aan onze filmnachten inclusief 10 things I hate about you. Riverdance tapdansers en zelfgemaakte tompouchen 😊 We hebben veel lief en leed gedeeld, ik ben onwijs blij met zo'n lieve en sterke vriendin als jij!
Pap en mam, hoe moet ik nu in woorden weergeven wat jullie voor me betekenen. Jullie zijn er altijd voor me, al bel ik midden in de nacht vanwege muizen in bed of moet ik in Leuven opgehaald worden met een pijnlijke rug. Zonder jullie was ik hier nooit gekomen, bedankt voor jullie onvoorwaardelijke steun en liefde.

Lieve, lieve Joost. Het was fijn om alle pieken en dalen van het promoveren met jou samen door te maken, en heel bijzonder dat we het nu ook echt samen afsluiten! Je hebt een grote bijdrage gehad bij het tot stand komen van dit boekje, zowel in mentale ondersteuning als praktische hulp bij websites, logo’s en online vragenlijsten (zonder jou was er waarschijnlijk nu nog geen data om op te schrijven.). Ik ben onwijs gelukkig met jou! En nu, samen op naar het volgende avontuur!
Eeske van Roekel was born on the 24th of January 1985 in Ede, The Netherlands. After completing secondary education (VWO) in 2003, she moved to Nijmegen to study Pedagogical Sciences at the Radboud University. In 2008, she graduated cum laude from the Research Master Behavioural Science. After graduation, she was asked to become the PhD candidate on a grant from the Convenant Radboud University Nijmegen - KU Leuven. Her PhD project focused on loneliness in adolescence. By using the Experience Sampling Method, she examined how lonely adolescents behaved in their day-to-day lives. In addition, she investigated the genetic basis of loneliness by conducting several gene-environment studies. Because her PhD project was based on a collaboration with Prof. Dr. Luc Goossens from the KU Leuven in Belgium, she spent a few months working at the Department of School Psychology and Child and Adolescent Development in Leuven. She presented her work at national and international conferences (e.g., Society for Research on Adolescence, Society for Research on Child Development), she was selected to participate in an international summer school on research in adolescence (EARA/SRA summer school), and she organized several workshop with internationally renowned scholars. In 2012, she received the Emerging Scholar Best Article Award from the Journal of Youth and Adolescence. Besides her research, Eeske taught several courses, for which she received the University Teaching Qualification (BKO).

At this moment, Eeske is working as a postdoctoral researcher at the Interdisciplinary Center Psychopathology and Emotion regulation of the University Medical Center in Groningen (UMCG). Her research focuses on loss of pleasure (i.e., anhedonia) in adolescence.