Loneliness: Cognitive factors and neurobiological mechanisms

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Table of Contents

List of abbreviations

AI	Anterior insula
SA	Social anxiety

1. Abstract

Loneliness is a painful condition with detrimental effects on physical and mental health. However, it is still unclear which mechanisms hinder lonely individuals from forming new social relationships which might alleviate their loneliness. The aim of the current thesis was thus to investigate potential factors contributing to the development or maintenance of loneliness. Specifically, alexithymia, a personality trait characterized by reduced emotional awareness, was examined as a predictor for loneliness. As alexithymia might lead to difficulties in forming new relationships during social transition phases, alexithymic traits of first-year students were assessed, and loneliness as well as perceived stress were monitored for six months during their first semester at university (study 1). Moreover, previous work indicated that the relationship between alexithymia and loneliness might be mediated by interpersonal trust, which is crucial not only for developing relationships but also for the beneficial effects of social interactions. Therefore, study 2 investigated to what extent reduced trust mechanistically contributes to impaired social interactions associated with loneliness. Ultimately, the hypothesized decreased emotional awareness and reduced interpersonal trust might promote social avoidance behavior, which might further foster loneliness. Hence, study 3 examined known behavioral and neural correlates of social avoidance in lonely individuals. The results of the current thesis provide evidence for loneliness as a vulnerability factor for increased stress experiences, which might drive the detrimental health effects of loneliness. Moreover, the predictive role of alexithymia for loneliness was confirmed, and diminished insular reactivity to emotional stimuli could be identified as a potential underlying mechanism of this relationship. Further support for the involvement of the anterior insula (AI) in loneliness was provided by study 2. As hypothesized, lonely individuals reported reduced interpersonal trust, and blunted AI functioning during trust decisions was associated with an attenuated affective and endocrinological responsiveness to a positive social interaction. A compromised neural integration of trust-related information might thus underlie impaired social interactions in loneliness. However, loneliness was not associated with correlates of social avoidance as known for social anxiety. Conclusively, the current thesis provides evidence for loneliness as a unique construct distinguishable from related psychopathology and offers important starting points for the development of scientifically based interventions.

2. Introduction and aims with references

Everybody knows what loneliness feels like. Everybody knows the pain resulting from feeling being left out, misunderstood, or just the feeling that you need somebody who helps you deal with everyday struggles. And everybody knows what helps to resolve this pain: turning to a good friend who gives you the feeling to belong. But what happens if the feeling of social isolation persists and the relationships you have are not sufficient – whether it is because they are too superficial or because there are too few of them?

The need to belong is a fundamental organizational principle of human behavior (Baumeister and Leary, 1995) with positive social relationships being crucial for health and survival (Holt-Lunstad et al., 2010). From an evolutionary perspective, loneliness, the perceived discrepancy between one's actual and desired social relationships regarding their quality or quantity (Perlman and Peplau, 1981), might thus have evolved as a distressful feeling to motivate the formation and maintenance of social relationships in the same way as hunger induces scavenging (Qualter et al., 2015). Indeed, the neural mechanisms involved in the craving for social cues after a short period of social isolation remarkably overlap with those involved in food craving after fasting (Tomova et al., 2020). Likewise, acute loneliness is associated with increased affiliative behavior (Reissmann et al., 2021). However, when the experience of loneliness becomes chronic, it has severe effects on physical and mental health (Quadt et al., 2020). For instance, loneliness increases the risk for psychological disorders (Mann et al., 2022), cardiovascular diseases (Valtorta et al., 2016), and all-cause mortality comparably to established risk factors such as obesity or substance abuse (Holt-Lunstad et al., 2010). Consequently, loneliness has been highlighted as a major public health challenge with high economic costs, emphasizing the urgent need for interventions targeting loneliness (Jeste et al., 2020). Nevertheless, developing effective interventions requires a thorough understanding of the etiological mechanisms that promote loneliness. While the detrimental health effects of loneliness are well-established, it is still elusive which mechanisms contribute to the development or maintenance of loneliness. Therefore, this thesis aims at investigating potential factors that may hinder lonely individuals from alleviating their loneliness. Specifically, since prolonged loneliness may be based precisely on the fact that social relationships are lacking or of low quality, the focus of this thesis lies on potential mechanisms which might particularly hamper the formation of new, positive relationships.

2.1. Factors contributing to loneliness: the potential role of alexithymia, interpersonal trust, and avoidance behavior

Social relationships can emerge throughout life, but changes in one's social network occur primarily as a result of major life events (Wrzus et al., 2013). As such, transition phases like the transition from school to university provide ample opportunities to make new friends through a new social environment but also hold the risk of harmful encounters. Thus, identifying events as stressful or safe is crucial to adequately deal with this uncertainty, whereas inadequate reactions, such as withdrawal from salutary social interactions or maintaining unhealthy relationships, could contribute to the persistence of loneliness. An impaired ability to successfully interpret potentially stressful events, however, has been hypothesized for individuals with high alexithymia due to a lack of emotional awareness characterizing this personality trait (Martin and Pihl, 1985). Accordingly, individuals with high alexithymia may have difficulty taking advantage of social transition phases to establish social relationships, suggesting that alexithymia might be an essential predictor of loneliness. Enhanced feelings of loneliness, in turn, could contribute to exacerbated experiences of psychosocial stress associated with alexithymia (Martin and Pihl, 1985). Indeed, a previous study indicated that alexithymia is associated with loneliness (Qualter et al., 2009), but its predictive role for prolonged loneliness remains unknown due to the cross-sectional study design. Interestingly, however, interpersonal trust could be identified as mediating factor between alexithymia and loneliness (Qualter et al., 2009). An impaired interpersonal trust in loneliness might be of great importance when it comes to the development of social relationships.

Positive relationships are mainly based on cooperation, but cooperative behavior requires interpersonal trust, especially during initial encounters when there is no information about the likelihood of reciprocity (Axelrod and Hamilton, 1981; Balliet and Van Lange, 2013). In contrast, lonely individuals might prefer self-centered safety behavior due to a default distrust (cf. Qualter et al., 2009). Self-centered, non-cooperative behavior, in turn, evokes avoidance or even punishment (Axelrod and Hamilton, 1981). Consequently, given the key role of trust for the development of social relationships and the beneficial effects of reciprocal positive social interactions, an impaired interpersonal trust might be an essential mechanism hindering lonely individuals to profit from social interactions. This way, even positive social interactions might fail to alleviate feelings of loneliness.

However, studies investigating whether an impaired interpersonal trust is mechanistically related to lower quality of social interactions in lonely individuals are lacking.

Notably, negative experiences of lonely individuals with social interactions may be generalized in the long run, reinforcing negative cognitive biases and consolidating avoidance behavior (Qualter et al., 2015). Hence, it might be promising to address avoidance behavior in addition to the introduced psychosocial or cognitive factors to break this vicious cycle. Evidence for social avoidance in loneliness stems not only from empirical observations of increased preferred interpersonal distances (Saporta et al., 2021) but also from recent findings highlighting a close link between loneliness and social anxiety (SA) (see, for example, Lim et al., 2016). This close link suggests that adapting established psychotherapies for SA could accelerate the development of interventions to reduce loneliness. Indeed, preliminary evidence indicates that established cognitive-behavioral treatments targeting SA decrease loneliness concurrently (O'Day et al., 2021). However, a better understanding of the shared underlying mechanisms of loneliness and SA is needed to improve therapeutic outcomes. The investigation of neurobiological factors might shed light on overlapping mechanisms involved in loneliness and SA, thereby facilitating the identification of promising therapeutic targets to reduce loneliness.

2.2. Neurobiological factors of loneliness

Various lines of research have linked loneliness with changes in the morphology and functioning of the amygdala (for comprehensive reviews, see Lam et al., 2021; Morr et al., 2022). These changes are particularly important given that threat-related amygdala hyperactivity is a core mechanism of SA and may predict avoidance behavior (Björkstrand et al., 2020; Brühl et al., 2014). Moreover, the neural responsiveness of the ventral striatum to social rewards seems to be reduced in both individuals suffering from loneliness and SA (Lam et al., 2021; Schultz et al., 2019), thereby providing further support for overlapping neurobiological mechanisms of loneliness and SA. Nevertheless, alterations in the amygdala and reward-related brain activity might reflect not only avoidance behavior and impaired responsiveness to social interactions but also an affected processing of trust decisions (Krueger and Meyer-Lindenberg, 2019). In line, further brain regions included in the neural circuit of trust seem to be associated with loneliness, including the medial prefrontal cortex, the temporoparietal junction, and the

anterior insula (AI) (Lam et al., 2021). Particularly, an affected trust processing in the AI might be important as the AI is not only recruited during trust decisions but is also associated with emotional awareness (Terasawa et al., 2013). An affected AI reactivity might thus contribute to the interplay of alexithymia, interpersonal trust, and loneliness. However, loneliness and alexithymia also share blunted responses to emotional stimuli in a larger limbic neurocircuitry including the amygdala and an affected reactivity of the anterior cingulate cortex (Lam et al., 2021; van der Velde et al., 2013). Moreover, inferences about cognitive processes from neural activation should always be drawn with restraint (Poldrack, 2006), especially given the low selectivity of activation of the mentioned brain regions, which are associated with various cognitive processes (Feng et al., 2021). Hence, it is still unclear to what extent neurobiological correlates of loneliness mechanistically underlie reduced emotional awareness, impaired trust processing, or social avoidance behavior.

2.3. Research aims

The aim of this thesis is the investigation of mechanisms that may promote the development or maintenance of loneliness. Therefore, three studies were conducted focusing on the association of loneliness with alexithymia, interpersonal trust, and social avoidance, given that these factors might particularly hamper the formation of new, positive relationships, as outlined above. Specifically, the following research questions were addressed:

(1) Do alexithymic traits predict loneliness during a transition phase from school to university, and does loneliness mediate the enhanced psychosocial stress experience associated with alexithymia? (Study 1)

(2) Is loneliness associated with reduced interpersonal trust, and are alterations in interpersonal trust related to a reduced benefit of new, positive social encounters in lonely individuals? (Study 2)

(3) Are behavioral and neural correlates of social avoidance known for SA shared by lonely individuals? (Study 3)

In all studies, functional magnetic resonance imaging was used to provide insights into the underlying neurobiological mechanisms of loneliness. This way, the results of the

current thesis might enable the identification of possible starting points for interventions to reduce loneliness.

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3. Publications

3.1. Publication 1: Insula reactivity mediates subjective isolation stress in alexithymia

Morr M*, **Lieberz J***, Dobbelstein M, Philipsen A, Hurlemann R, Scheele D. Insula reactivity mediates subjective isolation stress in alexithymia. Sci Rep 2021; 11: 15326. doi: https://doi.org/10.1038/s41598-021-94799-w. Impact factor: 4.997

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Insula reactivity mediates subjective isolation stress in alexithymia

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The risk for developing stress-related disorders is elevated in individuals with high alexithymia, a personality trait characterized by impaired emotional awareness and interpersonal relating. However, it is still unclear how alexithymia alters perceived psychosocial stress and which neurobiological substrates are mechanistically involved. To address this question, we examined freshmen during transition to university, given that this period entails psychosocial stress and frequently initiates psychopathology. Specifically, we used a functional magnetic resonance imaging emotional face matching task to probe emotional processing in 54 participants (39 women) at the beginning of the first year at university and 6 months later. Furthermore, we assessed alexithymia and monitored perceived psychosocial stress and loneliness via questionnaires for six consecutive months. Perceived psychosocial stress significantly increased over time and initial alexithymia predicted subjective stress experiences via enhanced loneliness. On the neural level, alexithymia was associated with lowered amygdala responses to emotional faces, while loneliness correlated with diminished reactivity in the anterior insular and anterior cingulate cortex. Furthermore, insula activity mediated the association between alexithymia and loneliness that predicted perceived psychosocial stress. Our findings are consistent with the notion that alexithymia exacerbates subjective stress via blunted insula reactivity and increased perception of social isolation.

Major life events such as the transition from school to university or from work to retirement involve changes in the social environment and are frequently accompanied by increased psychosocial stress[1]. The allostatic load[2], that is the wear and tear resulting from chronic overactivity of stress systems, can increase the risk of stress-related disorders like major depression or anxiety. Both environmental factors and interindividual differences modulate the allostatic load. Specifically, the ability to effectively cope with a life stressor is decreased in individuals with high alexithymia[3], a personality trait characterized by impaired emotional awareness and interpersonal relating. According to the stress-alexithymia hypothesis[4], the lack of emotional awareness hinders the identification of an event as stressful and the resulting ineffective coping aggravates the allostatic load. In fact, there is accumulating evidence that alexithymia has detrimental effects on mental and physical health[5–7]. In addition, alexithymia is associated with dysfunctional interpersonal bonding, which might lead to distressful feelings of loneliness if the quality or quantity of social relationships does not satisfy a person's need to belong[8,9]. Loneliness and social withdrawal in turn foster depressive symptomatology[10] and may increase the risk of relapse[11]. Furthermore, recent studies support close associations between loneliness, atypical physiological responses to acute stress and detrimental emotion-oriented stress coping strategies[12–15]. Collectively, not only the objective availability of support via social networks may modulate the allostatic load during transition phases, but also the subjective perception of social connectedness. Therefore, alexithymia might negatively impact psychological well-being and mental health via impaired interpersonal relating[16,17]. However, while the stress-alexithymia hypothesis is well established, it is still unclear whether alexithymia affects perceived stress during social transition phases by enhancing feelings of loneliness. Moreover, little is known about the underlying neurobiological mechanisms that promote the detrimental effects of alexithymia on stress responses.

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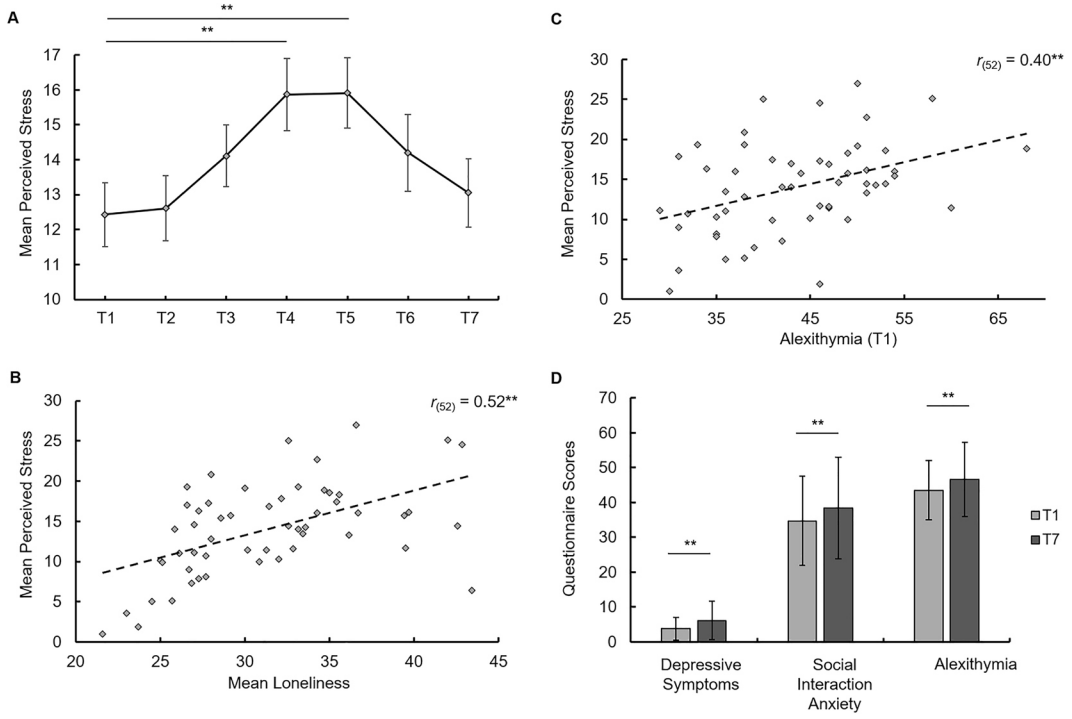

Figure 1. Perceived stress significantly changed over time and peaked in months four and five of the observation period (**A**). Mean perceived stress positively correlated with mean loneliness (**B**) and alexithymia (**C**) at study entry. Depressive symptoms, social interaction anxiety and alexithymia significantly increased after 6 months (**D**). Error bars show the standard error of the mean. $^{**}p < 0.01$, T1–T7, first to seventh month.

Importantly, alexithymia and loneliness seem to affect similar neural pathways: a meta-analysis of neuroimaging studies[18] revealed that high levels of alexithymia are associated with blunted responses to emotional stimuli (e.g. happy and fearful faces) in a limbic neurocircuitry including the amygdala and insular cortex and elevated responses in the anterior cingulate cortex (ACC) that may reflect difficulties in identifying and regulating emotions. Likewise, in highly lonely individuals, pleasant social stimuli elicited less activity in the striatum, amygdala, insula and ACC[19]. Of note, these brain regions have been identified as important neural hubs of stress resilience such that robust amygdala responses to emotional stimuli and functional coupling of ACC-insula circuitry might promote adaptive stress responses[20,21]. Moreover, a recent study demonstrated that targeted amygdala neurofeedback improves stress coping and reduces alexithymia[22], further strengthening the assumption that alexithymia and loneliness prevent favorable stress response by shared neural response patterns.

The current study thus aims to probe whether alexithymia might affect perceived stress by enhancing feelings of loneliness and to examine which neural substrates are involved. Therefore, we measured alexithymic traits and neural activation patterns in response to social stimuli (emotional faces) during functional magnetic resonance imaging (fMRI) in a sample consisting of 54 healthy freshmen. Participants were monitored during their first 6 months of the transition to university. Each month, participants completed questionnaires measuring their loneliness and their subjective stress experiences during this major life event. Specifically, we hypothesized a positive correlation between alexithymia and subjective stress response across time and that this relationship would be mediated by feelings of loneliness. Given the intertwined phenotype of alexithymia and loneliness as well as the overlapping neural correlates of both constructs, we predicted that both higher alexithymic traits and higher loneliness levels would be associated with altered responses to emotional face stimuli in the ACC, insula and amygdala. To this end, we used alexithymia and loneliness scores as continuous covariates in the analyses. Finally, we expected that the link between alexithymia and perceived stress would be mechanistically mediated by altered activity in these brain regions.

Results

Behavioral results. Stress levels changed significantly over time ($F_{(6,294)} = 4.56$, $p < 0.01$, $\eta_p^2 = 0.09$) and peaked in month four ($t_{(52)} = 4.48$, Bonferroni-corrected p (p_{cor}) < 0.01, $d = 0.47$) and five ($t_{(53)} = 3.92$, $p_{cor} = 0.02$, $d = 0.49$) of the observation period in comparison to the stress levels at study entry (see Fig. 1A), reflecting the first examination phase. In contrast, social network size ($F_{(6,282)} = 0.96$, $p = 0.43$, $\eta_p^2 = 0.02$) and loneliness scores ($F_{(6,288)} = 1.69$, $p = 0.14$, $\eta_p^2 = 0.03$) did not significantly change during the time course (see Table S1). As predicted, both the average loneliness ($r_{(52)} = 0.52$, $p < 0.01$; see Fig. 1B) and alexithymia in the first month (T1) positively correlated with the average perceived stress in the 6 months ($r_{(52)} = 0.40$, $p < 0.01$; see Fig. 1C), showing that individuals with greater dysfunctional emotional awareness and higher subjective lack of social con-

nection experienced more stress during the transition phase. In addition, T1 alexithymia positively correlated with psychosocial stress ($r_{(52)} = 0.49$, $p < 0.01$) already at study entry, but was not significantly associated with the increase in stress levels (i.e. maximum stress minus baseline) during the first examination phase ($p > 0.05$), indicating that alexithymia is associated with consistently increased perceived stress levels rather than increased acute stress responsiveness. Furthermore, depressive symptoms ($t_{(53)} = 3.19$, $p < 0.01$, $d = 0.53$), social interaction anxiety ($t_{(53)} = 3.05$ $p < 0.01$ $d = 0.26$) and alexithymia ($t_{(53)} = 2.83$, $p < 0.01$, $d = 0.32$) significantly increased after 6 months (see Fig. 1D).

fMRI task effects. Across both fMRI sessions, the participants exhibited increased responses to emotional faces (fearful and happy) compared to neutral ones in middle temporal regions (L (left): x, y, z coordinates of peak voxel in Montreal Neurological Institute space (MNI_{xyz}): − 60, − 56, 2, $k_E = 125$, after familywise error corrections (p_{FWE}) on cluster level $p_{FWE} = 0.02$; R (right): MNI_{xyz}: 58, − 58, 12, $k_E = 198$, $p_{FWE} < 0.01$), the inferior temporal gyrus (MNI_{xyz}: − 42, − 42, − 16, $k_E = 135$, $p_{FWE} = 0.01$) and middle occipital regions (L: MNI_{xyz}: − 22, − 98, 0, $k_E = 723$, $p_{FWE} < 0.01$; R: MNI_{xyz}: 26, − 90, 0, $k_E = 925$, $p_{FWE} < 0.01$). Furthermore, subjects showed stronger activation in response to fearful faces relative to neutral faces in clusters including the middle temporal gyrus (L: MNI_{xyz}: − 58, − 52, 4, $k_E = 293$, $p_{FWE} < 0.01$; R: MNI_{xyz}: 58, − 58, 14, $k_E = 533$, $p_{FWE} < 0.01$), the left inferior temporal gyrus (MNI_{xyz}: − 42, − 44, − 16, $k_E = 206$, $p_{FWE} < 0.01$), the right occipital region (MNI_{xyz}: 26, − 90, 0, $k_E = 589$, $p_{FWE} < 0.01$) and lingual areas in the left hemisphere (MNI_{xyz}: − 20, − 90, − 14, $k_E = 591$, $p_{FWE} < 0.01$). Moreover, subjects showed increased activity in middle occipital regions (L: MNI_{xyz}: − 20, − 98, 2, $k_E = 437$, $p_{FWE} < 0.01$; R: MNI_{xyz}: 32, − 92, 6, $k_E = 537$, $p_{FWE} < 0.01$) in response to happy faces compared to neutral ones. There were no significant whole-brain differences between the first (T1) and the seventh month (T7).

Correlation analyses of alexithymia and loneliness with brain activation. Individuals with high alexithymia showed decreased right amygdala responses to emotional faces in contrast to neutral faces at T1 (MNI_{xyz}: 34, 2, − 24, $t_{(53)} = 3.55$, $p_{FWE} = 0.03$ on peak level; see Fig. 2A). Furthermore, subjects with higher loneliness exhibited reduced activation in response to emotional faces in the left and right anterior insula (L: MNI_{xyz}: − 36, 12, 8, $t_{(53)} = 4.36$, $p_{FWE} = 0.02$; R: MNI_{xyz}: 48, 8, 4, $t_{(53)} = 4.21$, $p_{FWE} = 0.03$; see Fig. 2B), and ACC (L: MNI_{xyz}: 0, 28, 24, $t_{(53)} = 4.85$, $p_{FWE} < 0.01$; R: MNI_{xyz}: 2, 26, 24, $t_{(53)} = 4.82$, $p_{FWE} < 0.01$; see Fig. 2C) at T1. Likewise, loneliness negatively correlated with responses to fearful faces in the left anterior insular cortex (MNI_{xyz}: − 34, 10, 10, $t_{(53)} = 4.73$, $p_{FWE} = 0.01$) and ACC (L: MNI_{xyz}: 0, 8, 26, $t_{(53)} = 4.79$, $p_{FWE} = 0.01$; MNI_{xyz}: 0, 28, 24, $t_{(53)} = 4.70$, $p_{FWE} = 0.01$; R: MNI_{xyz}: 2, 26, 24, $t_{(53)} = 4.52$, $p_{FWE} = 0.01$; MNI_{xyz}: 2, 8, 28, $t_{(53)} = 4.03$, $p_{FWE} = 0.03$) and anterior insula responses (MNI_{xyz}: 34, 12, 4, $t_{(53)} = 4.12$, $p_{FWE} = 0.04$) to happy faces. These associations were not evident at T7.

Mediation analysis. To examine whether higher levels of alexithymia predicted perceived stress levels by enhancing feelings of loneliness, a first mediation analysis was calculated with alexithymia as predictor for subjective stress and loneliness as mediator. A significant mediation via loneliness [indirect effect of alexithymia on stress via loneliness: $\beta = 0.20$, standard error (SE) = 0.10, 95% confidence interval (CI) 0.04–0.43] indicated that the detrimental effects of alexithymia on perceived stress were indeed mediated by loneliness with the direct effect of alexithymia on stress being diminished after including loneliness (total effect of alexithymia on stress: $\beta = 0.40$, $p = 0.003$, SE = 0.13, 95% CI 0.15–0.66; direct effect of alexithymia on stress after including loneliness as mediator: $\beta = 0.20$, $p = 0.14$, SE = 0.13, 95% CI −0.07 to 0.47). In a second step, we added the parameter estimates of the right amygdala, right ACC and right anterior insula as further mediator variables to the model to elucidate the underlying neural mechanisms. For each brain region, two models were calculated to test potential mediation effects on all pathways (i.e., both serial and parallel mediation effects were tested). The analyses revealed that the link between alexithymia and loneliness was driven by reduced insula reactivity, leading to a significant indirect effect of alexithymia on stress via insula reactivity and loneliness (serial mediation: $\beta = 0.06$, SE = 0.04, 95% CI 0.01–0.15, see Fig. 3). Specifically, alexithymia predicted the reduced anterior insula reactivity which was linked to enhanced feelings of loneliness which in turn, predicted subjective stress. This mediation was mainly driven by the Toronto Alexithymia Scale (TAS) factors "difficulties describing feelings" (DDF) and "difficulties identifying feelings" (DIF) (see SI Results). No further mediation effects were observed for the insula, amygdala or ACC (all 95% CIs of further indirect effects via brain activation included zero).

Discussion

The present study aimed at elucidating the neural mechanisms moderating the link between alexithymic traits, loneliness and stress reactivity during the transition to university. Our results confirmed that loneliness mediated the noxious association between alexithymia and subjective stress during the first 6 months of university. Moreover, we found that the anterior insula plays a crucial role in this process, by mediating the link between alexithymia and loneliness.

Our results provide further support for and extend the stress-alexithymia hypothesis[4]. We were able to replicate previous models suggesting a close link between alexithymia and loneliness[11] and found that individuals with high alexithymia, especially with difficulties in describing and identifying emotions, experience more stress during transition phases partly because they perceive more subjective social isolation. This finding is consistent with previous studies reporting significant associations between the TAS DIF and DDF subscales and loneliness[23] as well as a relationship between the TAS DIF subscale and poor adjustment during transition to university and perceived stress[24]. Intriguingly, our results indicate that this mechanism may be driven by diminished insula responses to emotional signals which directly link alexithymia with loneliness. The insular cortex is a hub for interoceptive processing and conscious affect[25] and endotoxin-induced changes in the glucose metabolism of

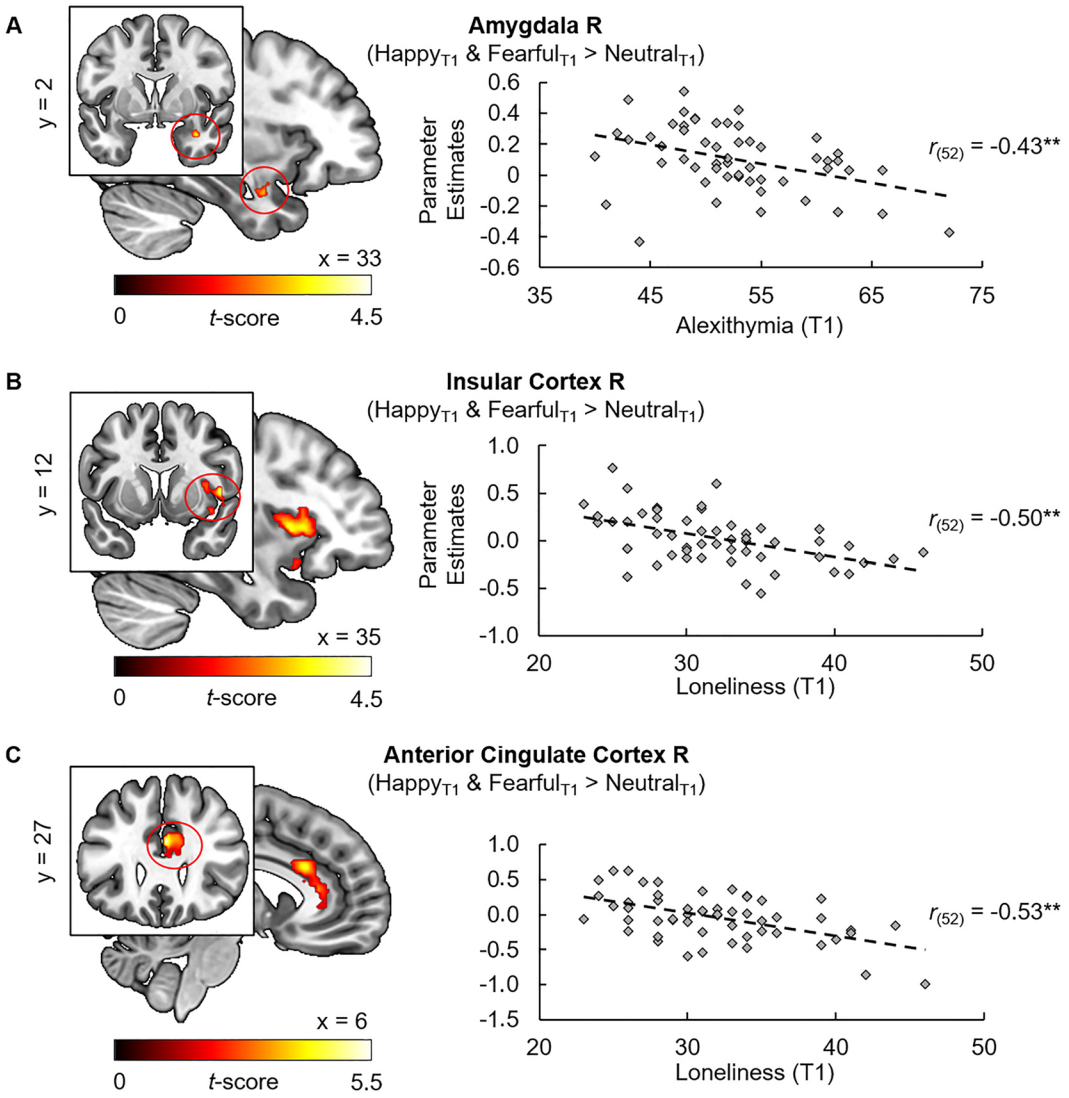

Figure 2. Participants with high alexithymia showed reduced activation to emotional faces compared to neural faces in the right amygdala (**A**; MNI_{xyz}: 34, 2, − 24, $t_{(53)} = 3.55$, $p_{FWE} = 0.03$). Individuals with high loneliness exhibited lower responses to emotional faces in the right anterior insular cortex (**B**; MNI_{xyz}: 48, 8, 4, $t_{(53)} = 4.21$, $p_{FWE} = 0.03$) and the right anterior cingulate cortex (**C**; MNI_{xyz}: 2, 26, 24, $t_{(53)} = 4.82$, $p_{FWE} < 0.01$). For illustration purpose clusters are shown with significance level of $p = 0.05$. $^{**}p < 0.01$, *FWE* familywise error corrected, *L* left, *MNI* Montreal Neurological Institute, *R* right, *T1* study entry.

the right insula positively correlate with changes in social interest[26]. Likewise, individuals with high loneliness have been found to exhibit reduced insula responses during interpersonal trust decisions[27]. Moreover, multiple lines of evidence indicate that insula pathology leads to alexithymia. For instance, dopamine D2-type receptor availability in the insula has been linked to higher alexithymia[28], the gray matter volume of the insular cortex inversely correlated with alexithymia[29] and the extent of damage to the anterior insula predicted alexithymia in lesion patients[30]. It has been theorized that insula dysfunction in alexithymia may reflect a transdiagnostic marker of empathic deficits[31] and our findings in healthy participants point to an additional mechanism such that the dampened insula responses to external emotional cues underlie the association of alexithymia with enhanced perceived social isolation. Along these lines, the observed pattern of results is consistent with the notion that social connectedness requires the ability to flexibly shift between interoceptive and exteroceptive attention[32] which may be based on recruitment of the anterior insula.

Furthermore, consistent with previous fMRI studies[18,19], we found decreased amygdala and ACC responses in individuals with high alexithymia and loneliness, respectively. The amygdala has often been linked to alexithymia[18,33] and a recent study showed that neurofeedback targeting the amygdala during military training not only enhanced stress coping but also decreased alexithymia[22]. Moreover, the amygdala has also been linked to loneliness and social support. For example, a decrease in perceived stress and loneliness was moderated by amygdala volumes[34] and the experience of social support was regulated by amygdala activity[35]. Likewise, the ACC

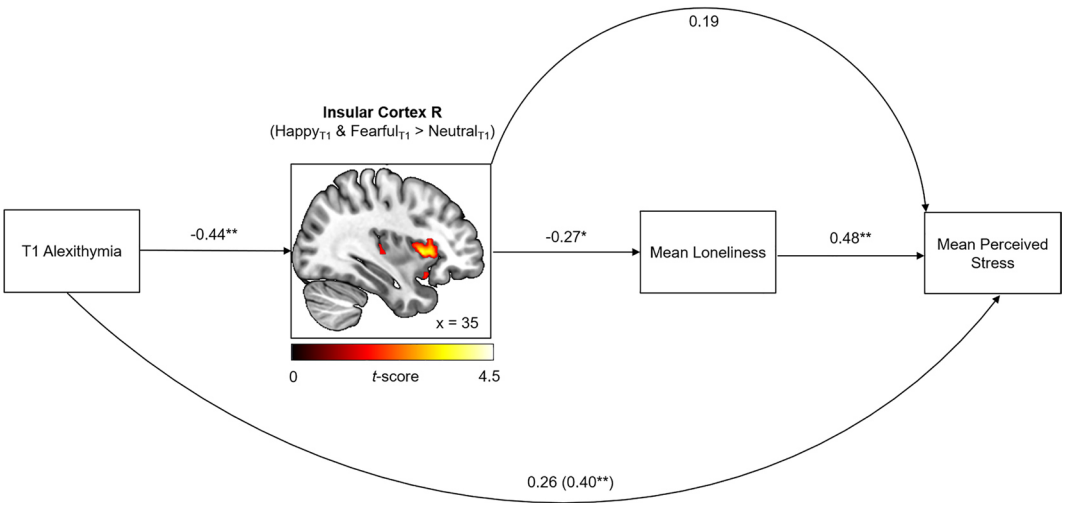

Figure 3. Mean loneliness mediated the relationship between alexithymia at study entry and mean perceived stress ratings. Furthermore, activation of the right insula in response to emotional stimuli at study entry mediated the link between alexithymia and loneliness. Numbers show standardized β coefficients. The β coefficient in brackets shows the total effect without mediators. Insula coordinates are shown in Montreal Neurological Institute space. For illustration purpose, the cluster is shown with a significance level of $p = 0.05$. $^{*}p < 0.05$, $^{**}p < 0.01$, *T1* study entry, *R* right.

has been previously linked not only to loneliness but also to alexithymia: ACC size correlates with alexithymia ratings especially in men[36] and high levels of alexithymia are associated with elevated responses to emotional stimuli in the ACC[18]. Furthermore, the ACC plays a role in social pain processing during social support[37] and overall seems to be a hub for the integration of social information and empathy[38]. Bearing in mind that neither ACC nor amygdala reactivity mediated effects of alexithymia, the insular cortex seems to be a crucial neural processing hub for the interplay between loneliness and alexithymia. Therefore, neurofeedback training targeting insula activation could lead not only to reduced feelings of loneliness but also to reduced psychosocial stress[22]. In contrast to loneliness, objective social network indices were not significantly associated with alexithymia or perceived stress. Given that in a previous study with college freshmen[39] psychological stress selectively mediated the association between antibody response to the influenza immunization and loneliness, but not social network size and immunization response, our data provide further support for the notion that the subjective perception of social connectedness may be a more important predictor for stress reactivity during transition phases than the objectively available social contacts.

Interestingly, the trait-dependent reactivity was no longer evident in the second fMRI session 6 months later, indicating either repetition effects and reduced retest-reliability or that a disrupted plasticity as observed in the prefrontal cortex with an attention-shifting task following long-term psychosocial stress[40] is more pronounced for limbic reactivity to emotional stimuli. Furthermore, we observed an increase in alexithymia scores, potentially elicited by the prolonged subjective stress, which might reflect an acquired secondary alexithymia[41]. As such, these experience-based changes may have masked genuine trait associations in the second fMRI session. Of note, the allostatic load of the transition to university caused a significant increase in depressive symptoms, social interaction anxiety and autistic-like traits after 6 months, thus illustrating that individuals with high alexithymia and loneliness might be at risk not only for poor academic performance but also stress-related psychological disorders due to chronically increased stress levels.

Collectively, our results provide evidence for a close interplay between emotional awareness and perceived social isolation, with dampened insula reactivity serving as a potential underlying mechanism linking alexithymia with loneliness and thus exacerbating the susceptibility to perceived stress. Based on these findings, neurobiologically-informed interventions with cognitive bias modification procedures should target the feeling of social disconnectedness to help students with alexithymic traits to better cope with psychosocial stress during transition phases. Furthermore, neurofeedback training targeting the insula might reduce the feeling of social isolation and therefore potentially enhance stress coping during stressful life events.

Methods

Subjects. Sixty healthy freshman students participated in the study after giving written informed consent. The study was approved by the institutional review board of the medical faculty of the University of Bonn and carried out in compliance with the latest revision of the Declaration of Helsinki. Subjects were screened prior to the first test session and received monetary compensation for study participation. Subjects had no past or present physical or psychiatric illness, as assessed by a medical history questionnaire and the Mini-International Neuropsychiatric Interview[42]. All subjects started their first semester without ever attending university courses before. Six subjects had to be excluded because they missed the second fMRI appointment (n = 3), showed excessive head motion in the MRI (> 3 mm/°; n = 2) or because of technical failures (n = 1). Therefore, final analyses

include data from 54 healthy freshman (39 women, mean age: 18.85 ± 0.88 years minimum [min]/maximum [max] age: 18/22; alexithymia: 45.06 ± 8.79, min/max: 25/68; loneliness: 31.30 ± 5.44, min/max: 20/54). Four subjects missed one of their monthly appointments resulting in data loss of 1.48%.

Experimental design. Subjects were monitored during their first semester at university for a total duration of 6 months, starting with a screening session in their first university month. Shortly (average: 14 days, min/max = 0/32 days) after the screening session, a first fMRI session was conducted (T1 = first month). The fMRI measurements were repeated after 6 months (T7 = seventh month; time between the two fMRI measurements = 164 days, min/max = 153/197 days). Participants completed several questionnaires every month between the two fMRI sessions measuring perceived stress, loneliness and social network size (see Fig. S1).

Questionnaires. Subjects completed different sets of questionnaires to continuously monitor social behavior during their first semester. In the screening session and before the second fMRI scan, we assessed alexithymia (TAS [Toronto Alexithymia Scale][43]), loneliness (UCLA LS [UCLA Loneliness Scale][44]) and perceived stress (PSS-10 [Perceived Stress Scale][45]). Furthermore, we monitored psychiatric symptoms during the transition phase by measuring social interaction anxiety (SIAS [Social Interaction Anxiety Scale][46]), social anxiety (LSAS [Liebowitz Social Anxiety Scale][47]), general trust (GTS [Yamagishi General Trust Scale][48]), autistic-like traits (AQ [Autism Spectrum Quotient][49]), depression symptoms (BDI [Becks Depression Inventory][50]) and trait anxiety (STAI [State Trait Anxiety Inventory][51]). Moreover, to differentiate between subjectively perceived social isolation (i.e. loneliness) and objective social network indices, we included the Social Network Size Questionnaire (SNS)[52]. We further assessed social support (F-SozU [Fragebogen zur Sozialen Unterstützung, short version K-14][53]) as a key resilience factor during transition phases, to further distinguish between perceived social isolation and perceived social support. Every month between these sessions, subjects completed the PSS-10, UCLA LS and SNS. For a detailed description of the TAS, UCLA LS and PSS-10, see SI Methods.

fMRI data acquisition. At the start of the experiment, subjects were instructed to lay as calm as possible. Functional data were acquired with a 3 T Siemens TRIO MRI system (Siemens AG, Erlangen, Germany) with a Siemens 32-channel head coil and obtained by using a T2*-weighted echoplanar (EPI) sequence [TR = 2690 ms, echo time (TE) = 30 ms, ascending slicing, matrix size: 96 × 96, voxel size: 2 × 2 × 3 mm^3, slice thickness = 3.0 mm, distance factor = 10%, field of view (FoV) = 192 mm, flip angle 90°, 41 axial slices]. High-resolution T1-weighted structural images were collected on the same scanner (TR = 1660 ms, TE = 2.54 ms, matrix size: 256 × 256, voxel size: 0.8 × 0.8 × 0.8 mm^3, slice thickness = 0.8 mm, FoV = 256 mm, flip angle = 9°, 208 sagittal slices). To control for inhomogeneity of the magnetic field, fieldmaps were obtained for each T2*-weighted EPI sequence [TR = 392 ms, TE (1) = 4.92, TE (2) = 7.38, matrix size: 64 × 64, voxel size: 3 × 3 × 3 mm^3, slice thickness = 3.0 mm, distance factor = 10%, FoV = 192 mm, flip angle 60°, 37 axial slices].

fMRI task. During the fMRI, subjects completed a well-established emotional face-matching paradigm[54,55]. To ensure the subjects' attention, subjects had to match the identity of two simultaneously presented pictures at the bottom of the screen with a target picture presented at the top. Stimuli consisted of face pictures (neutral, fearful and happy) and houses as non-social control stimuli. Stimuli were presented with Presentation 14 software (Neurobehavioral Systems, Albany, CA, USA) in three blocks for every condition (Happy, Fearful, Neutral, House) with each block consisting of five trials. Stimuli did not vary in emotional expression or in sociality during a block. Trial duration was 5 s with a 10 s pause after each block. In this pause, a fixation-cross was depicted. The identity of the face stimuli varied between T1 and T7 to reduce habituation effects. Participants could choose their responses using an MRI-compatible response grip system (NordicNeuroLab AS, Bergen, Norway). Responses and reaction times (RTs) were measured to evaluate possible attention effects. High-resolution anatomical images were acquired after the functional images.

fMRI analysis. The fMRI data were pre-processed and analyzed using standard procedures in SPM12 (Wellcome Trust Center for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab (The MathWorks Inc., Natick, MA). Participants with excessive head movements (> 3 mm/° in any direction, n = 2) or missing data due to technical failures (n = 1) were excluded from fMRI analyses. The first five volumes of each functional time series were discarded to allow for T1 equilibration. Functional images were corrected for head movements between scans by an affine registration. Images were initially realigned to the first image of the time series before being re-realigned to the mean of all images. To correct for signal distortion based on B0-field inhomogeneity, the images were unwarped by applying the voxel displacement map (VDM file) to the EPI time series (Realign & Unwarp). Normalization parameters were determined by segmentation and non-linear warping of the structural scan to reference tissue probability maps in MNI space. Normalization parameters were then applied to all functional images, which were resampled at 2 × 2 × 2 mm^3 voxel size. For spatial smoothing, a 6-mm full width at half maximum Gaussian kernel was used. Raw time series were detrended using a high-pass filter (cut-off period 128 s).

A two-stage approach based on the general linear model implemented in SPM12 implemented in Matlab (The MathWorks Inc., Natick, MA, USA) was used for statistical analyses. On the first level, participants' individual data were modelled using a fixed-effect model. Onsets and durations of the four experimental condition blocks ('Happy, 'Fearful', 'Neutral', 'House') were modelled by a boxcar function convolved with a hemodynamic response function (HRF). Movement parameters were included in the design matrix as confounds. On the second-level, main contrasts of interest [$Fearful_{First}$ > $Neutral_{First}$; $Happy_{First}$ > $Neutral_{First}$; $Fearful_{Second}$ > $Neutral_{Second}$; $Happy_{Second}$ > $Neutral_{Second}$; $Happy_{First}$ and $Fearful_{First}$ > $Neutral_{First}$; $Happy_{Second}$ and $Fearful_{Second}$ > $Neutral_{Second}$; Happy

$_{First > Second}$ and Fearful $_{First > Second}$ > Neutral $_{First > Second}$; Happy $_{First \& Second}$ and Fearful $_{First \& Second}$ > Neutral $_{First \& Second}$] were computed using one sample t-tests. Loneliness and alexithymia ratings were used as covariates for the second level analysis. The following whole-brain analysis was done with a height threshold of $p < 0.001$. The main analyses of fMRI data focused on regions of interests (ROIs) associated with emotion processing in alexithymia and loneliness consisting of the amygdala, ACC and insular cortex[18]. These ROIs were anatomically defined according to the Wake Forest University PickAtlas (wfu PickAtlas) for both hemispheres. Parameter estimates of significant ROI clusters were extracted using MarsBaR (http://marsbar.sourceforge.net) and further analyzed in SPSS 25 (IBM Corp., Armonk, NY, USA).

Statistical analyses. Repeated measures analyses of variance (ANOVAs) and Bonferroni corrected post-hoc *t*-tests were calculated using SPSS 25 (IBM Corp., Armonk, NY, USA) to examine changes in stress, loneliness and social network size over time. If the assumption of sphericity was significantly violated as assessed by Mauchly's tests, Greenhouse Geisser corrections were applied. Pearson correlations between parameter estimates of significant ROI clusters, loneliness, perceived stress and alexithymia were calculated. Furthermore, mediation analyses were carried out using the PROCESS macro v3.4 for SPSS[56]. Focusing on mean stress as outcome variable, we used T1 alexithymia as predictor variable and mean loneliness ratings as mediator. As we were interested in the neurobiological mechanisms underlying the link between alexithymia, loneliness and perceived stress, we also tested the hypothesized mediation effects of the neural correlates of alexithymia and loneliness. Parameter estimates of significant clusters associated with alexithymia or loneliness at the first fMRI session were thus included as additional mediator variables and mediation effects were tested for each pathway between the former mentioned behavioral results. For all mediation analyses, 10,000 bootstraps samples were used.

Data availability

The data that support the findings of the present study are openly available in the repository of the Open Science Foundation at https://osf.io/csn5u/?view_only=21e52c0df9e14712894596967c4511bc.

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Author contributions

M.M., J.L. and D.S. designed the experiment; M.M., J.L. and M.D. conducted the experiments; M.M., J.L., M.D. and D.S. analyzed the data. All authors wrote the manuscript. All authors read and approved the manuscript in its current version.

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Competing interests

The authors declare no competing interests.

Additional information

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3.2. Publication 2: Loneliness and the social brain: how perceived social isolation impairs human interactions

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RESEARCH ARTICLE

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Loneliness and the Social Brain: How Perceived Social Isolation Impairs Human Interactions

Jana Lieberz, Simone G. Shamay-Tsoory, Nira Saporta, Timo Esser, Ekaterina Kuskova, Birgit Stoffel-Wagner, René Hurlemann, and Dirk Scheele**

Loneliness is a painful condition associated with increased risk for premature mortality. The formation of new, positive social relationships can alleviate feelings of loneliness, but requires rapid trustworthiness decisions during initial encounters and it is still unclear how loneliness hinders interpersonal trust. Here, a multimodal approach including behavioral, psychophysiological, hormonal, and neuroimaging measurements is used to probe a trust-based mechanism underlying impaired social interactions in loneliness. Pre-stratified healthy individuals with high loneliness scores ($n = 42$ out of a screened sample of 3678 adults) show reduced oxytocinergic and affective responsiveness to a positive conversation, report less interpersonal trust, and prefer larger social distances compared to controls ($n = 40$). Moreover, lonely individuals are rated as less trustworthy compared to controls and identified by the blinded confederate better than chance. During initial trust decisions, lonely individuals exhibit attenuated limbic and striatal activation and blunted functional connectivity between the anterior insula and occipitoparietal regions, which correlates with the diminished affective responsiveness to the positive social interaction. This neural response pattern is not mediated by loneliness-associated psychological symptoms. Thus, the results indicate compromised integration of trust-related information as a shared neurobiological component in loneliness, yielding a reciprocally reinforced trust bias in social dyads.

1. Introduction

Humans are an essentially social species with the motivation to form and maintain interpersonal relationships as a fundamental organizational principle of behavior. When a person's need to belong is not satisfied, distressful feelings of loneliness, that is perceived social isolation, occur. Various lines of research indicate that loneliness has detrimental effects on mental and physical health, evident in increased risk of psychological disorders, cognitive decline, and all-cause mortality.[1,2] As such, loneliness has been identified as a public health challenge with prevalence rates up to 33% across age,[3] but the unclear etiological mechanisms leading to and fostering the maintenance of loneliness hamper the development of neurobiologically-informed interventions not only on the individual but also the societal level.[4–6]

From an evolutionary perspective, loneliness may have evolved to motivate the formation of new social relationships, in the same way as hunger induces scavenging.[7–9] However, when the connection with other individuals fails, loneliness impairs inflammatory and immune responses[6,10] and promotes a phenotypic hypersensitivity to social threats and self-centered behavior.[8,11] The perception of the social environment as threatening may lead to various negative biases in loneliness. For instance, it has been suggested that lonely individuals allocate their attention faster toward threatening social stimuli, anticipate

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rejection more often, and exhibit negative attribution styles.[11] Eventually, even positive social interactions might fail to alleviate feelings of loneliness, as lonely individuals show reduced positive ratings of social encounters and attenuated reward-associated brain activity in response to positive social stimuli.[12,13] Importantly, however, while the detrimental impact of loneliness on social interactions is well established and theoretical frameworks point to negative biases and selfish behavior as putative mediators, the neurobiological mechanisms that hinder the formation of new, positive relationships and thus the alleviation of loneliness are still elusive.

In human societies, the development of positive relationships is based mainly on cooperation, with non-cooperative behaviors evoking avoidance or even punishment. However, during initial encounters, when there is no prior information about the likelihood of reciprocity, rapid trustworthiness decisions are required for the formation of new relationships. Importantly, preliminary evidence indicates that interpersonal trust is reduced in lonely individuals.[11] In addition, the neural circuits of trust and loneliness are largely intertwined and share neuroanatomical pathways via the amygdala, the anterior insula (AI), the medial prefrontal cortex (mPFC), the nucleus accumbens (NAcc), and the temporoparietal junction (TPJ).[6,14–17] Nevertheless, it is still unclear whether these brain regions might contribute to reduced interpersonal trust in loneliness, as they have been associated with various cognitive processes.[18,19] This would indicate that the selectivity of activation is low and specific inferences are not valid without evidence that the assumed process (i.e., interpersonal trust) is engaged.[20]

Thus, the current study aims to examine to what extent loneliness relates to interpersonal trust, and whether activity and connectivity of the aforementioned neural circuit would be altered in lonely individuals during situations that specifically require trustworthiness decisions. We hypothesized that participants with high loneliness scores (high-lonely, HL) would exhibit diminished interpersonal trust in self-report and behavioral measurements as well as altered trust-associated brain activity and connectivity. Furthermore, given the key role of interpersonal trust for the development of positive relationships, we hypothesized that reduced interpersonal trust and its underlying brain activity would mechanistically contribute to attenuated benefits from a positive social interaction in lonely individuals.

To test our hypotheses, we implemented a multimodal prestratification approach including behavioral, psychophysiological, hormonal, and neuroimaging measurements. We screened a sample of $n = 3678$ individuals and included $n = 42$ HL and $n = 40$ controls (low-lonely, LL) who participated in a positive conversation with an unfamiliar confederate and underwent functional magnetic resonance imaging (fMRI) during which they played an adapted version of the well-established trust game.[21] Specifically, we hypothesized increased positive and decreased negative mood ratings as response to the positive conversation across all participants. Moreover, we expected that affective responses would be reduced in HL participants. In contrast, we hypothesized that HL and LL participants would not differ regarding their physiological responsiveness to the conversation (i.e., changes in electrodermal activity (EDA) and heart rate), as we assumed that the reduced affective responsiveness would be based on negative biases rather than differences in physiological arousal.

To probe the hypotheses of reduced interpersonal trust and trust-associated brain activity as a potential mechanism underlying the impaired reactivity to social interactions in loneliness, we first measured self-reported interpersonal trust and the ideal and uncomfortable interpersonal distance during a stop-approach paradigm[22] as behavioral measurement of interpersonal trust toward the confederate. We then contrasted brain activity during the fMRI trust game with a risk game control condition to test the hypothesized altered brain activity in the amgydala, AI, mPFC, NAcc, and TPJ and to further explore whether differences in brain activity would be accompanied by altered functional connectivity. We lastly hypothesized that the observed differences in responsiveness to the positive social interaction of HL compared to LL participants would correlate with the trust assessments. We controlled for the influence of possible confounding variables such as depressive symptomatology, social anxiety, and childhood maltreatment.

In addition to these hypotheses, we assessed further exploratory variables to better characterize the response profile to the positive social interaction in lonely individuals. We collected saliva samples before and after the task to explore hormonal and immunological reactivity. Salivary assessments consisted of the hypothalamic peptide oxytocin, which is crucially involved in human bonding and trust,[23–25] as well as cortisol and immunoglobulin A (IgA) concentrations as markers of stress and immune system responses[26] to the social interaction, in addition to baseline immune parameters in blood. Moreover, the blinded confederate in the social interaction task estimated the group affiliation (HL vs LL) and rated the trustworthiness of the participants to examine the social transmission of loneliness. Finally, as sex differences in the neural correlates of loneliness have been identified recently,[14] we conducted moderator analyses to explore the potential influence of the participants' sex on loneliness effects in our sample.

2. Results

2.1. Loneliness and Impaired Social Interaction

First, we examined behavioral, hormonal, and psychophysiological responses to a positive, real-life social interaction in a controlled setting. As expected, across groups (HL: $n = 42$, 21 female; LL: $n = 40$, 20 female, cf. Table S1, Supporting Information), the positive interaction was experienced as very pleasant [M ± SD: 82.19 ± 16.73 on a visual analogue scale (VAS) ranging from 0 ("not pleasant at all") to 100 ("very pleasant"); see **Figure 1**A] and significantly increased positive mood: specifically, we observed an increase in positive affect and in vigor [for all main effects of time (before vs after the interaction): Fs > 11.06, ps < 0.002, $\eta_p^2 > 0.12$; 95% confidence interval (CI) of increase in scores of the positive affect: 1.25 to 3.30; vigor: 0.96 to 3.85]. An increase in general physiological activity was evident for the skin conductance level (SCL) and heart beats per minute (BPM) (main effect of time for SCL: $F_{(1,72)} = 5.89$, $p = 0.018$, $\eta_p^2 = 0.08$, 95% CI of increase: 2.10 to 2.88 µS, see Figure 1B; BPM: $F_{(1,70)} = 11.36$, $p = 0.001$, $\eta_p^2 = 0.14$, 95% CI: 3.56 to 5.47 BPM, see Figure 1C). Furthermore, the positive social interaction led to elevated salivary oxytocin and IgA levels [area under the curve (AUC_I) describing the increase tested against zero: all ts > 2.59, ps < 0.013, ds > 0.38;

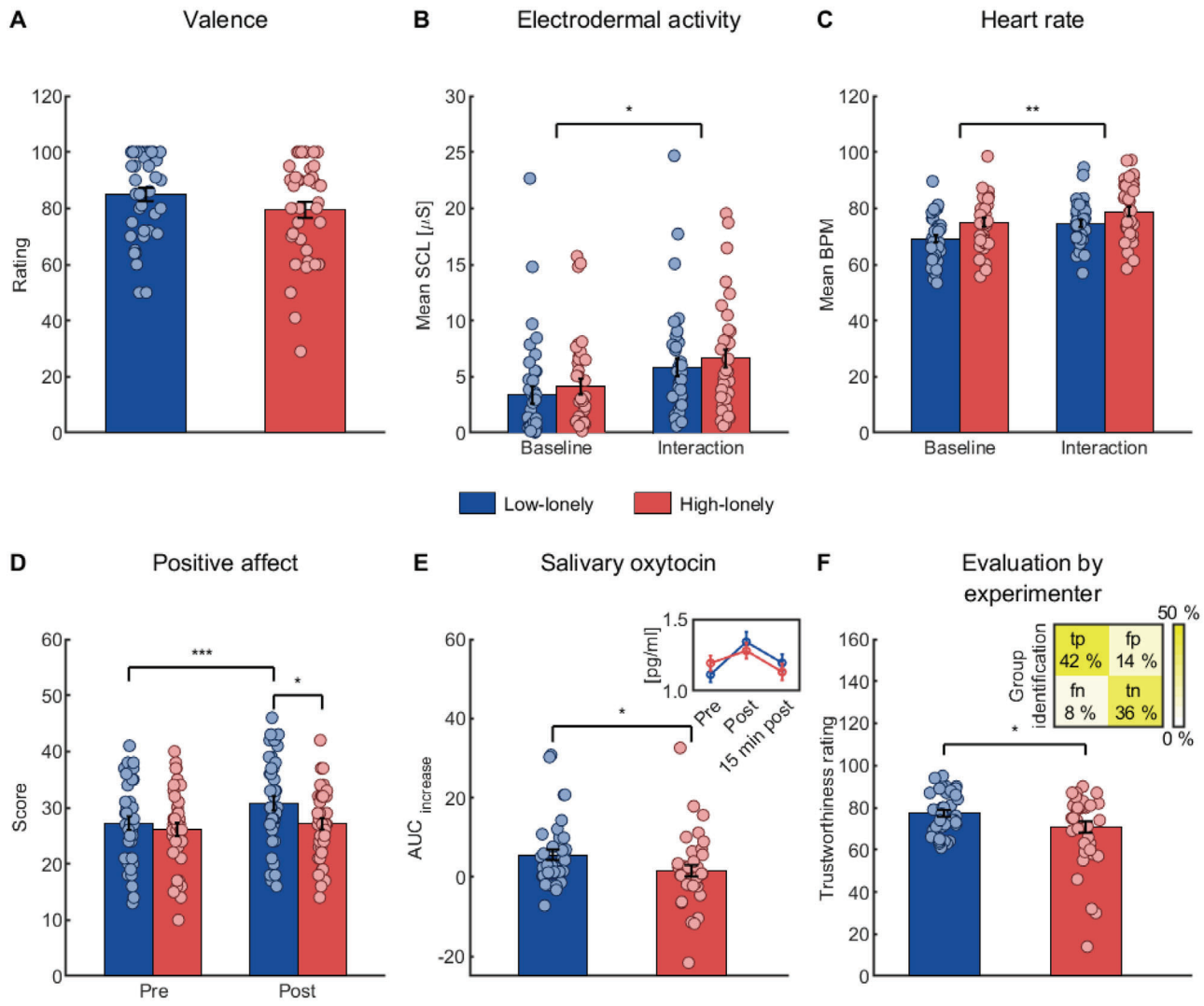

Figure 1. Response profile to the positive social interaction paradigm. A) Participants rated the positive social interaction as very pleasant on a visual analogue scale (VAS) ranging from 0 ("not pleasant at all") to 100 ("very pleasant") and ratings did not differ between groups. B) Across groups, mean skin conductance level (SCL) and C) mean heart beats per minute (BPM) increased during the social interaction compared to a 5-min rest baseline. However, high-lonely (HL) participants showed diminished reactivity to the social interaction. D) Positive affect increased in low-lonely (LL) but not HL participants and E) the area under the curve (AUC) measuring the increase in salivary oxytocin levels was attenuated in the HL sample. The inlay displays the group mean salivary oxytocin concentration for each time point. F) After completion of the social interaction, the experimenter rated HL participants as less trustworthy on a VAS ranging from 0 ("not trustworthy at all") to 100 ("very trustworthy") and identified HL participants significantly better than by chance. The inlay displays the percentage of false negative (fn; HL classified as LL), false positive (fp; LL classified as HL), true negative (tn; LL classified as LL), and true positive (tp; HL classified as HL) classifications. All bars represent group means. Error bars indicate standard errors of the mean. Dots are jittered for purposes of presentation. *p*-values were calculated using two-sample *t*-tests (A, $n = 79$; E, $n = 77$; F, $n = 78$), mixed analyses of variance (B, $n = 75$; C, $n = 73$), and post-hoc two-sample and paired *t*-tests (D, $n = 79$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

95% CI of AUC_I of oxytocin: 1.63 to 5.62; IgA: 0.06 to 0.51; mean percentage increase between before and after the interaction ± SD in oxytocin: 17.03 ± 31.23%; IgA: 16.21 ± 47.52%].

Importantly, as hypothesized, HL participants exhibited attenuated self-reported affective reactivity to the positive interaction (interaction of time and group for positive affect: $F_{(1,77)} = 6.43$, $p = 0.013$, $\eta_p^2 = 0.08$). Post-hoc *t*-tests revealed a significant increase in positive affect in LL participants [$t_{(39)} = 5.02$, $p < 0.0001$ after Bonferroni-correction (p_{cor}), $d = 0.45$, 95% CI of increase in score: 2.10 to 4.95], but not in HL participants ($t_{(38)} = 1.42$, $p_{cor} = 0.658$, 95% CI: −0.43 to 2.43, see Figure 1D). By contrast, the physiological reactivity to the positive social interaction did not differ between groups (no significant interaction of time with group for SCL or BPM measurements, all *p*s > 0.075), suggesting that observed affective group effects were not based on differences in the experiences of physiological arousal.

Interestingly, we did not observe baseline differences in plasma ($t_{(77)} = 0.13$, $p = 0.895$, 95% CI of group difference: −0.42 to 0.48 pg mL^{-1}) or salivary oxytocin levels ($t_{(76)} = 1.09$, $p = 0.278$, 95% CI: −0.07 to 0.23 pg mL^{-1}), but HL participants showed a reduced increase in salivary oxytocin levels compared to LL participants ($t_{(75)} = -2.04$, $p = 0.045$, $d = -0.47$, 95% CI of AUC_I

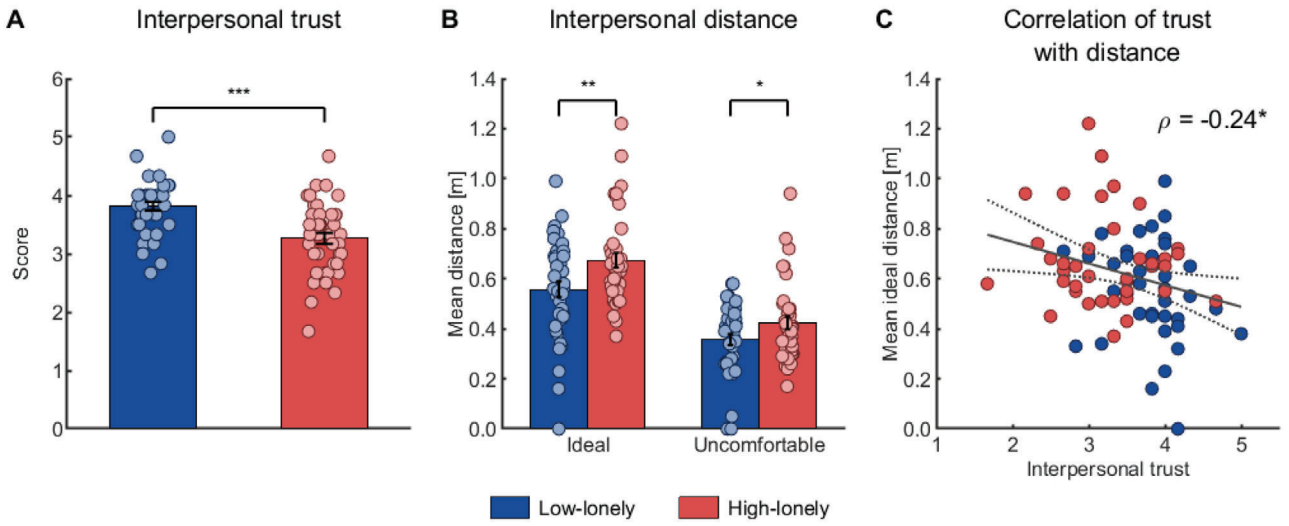

Figure 2. Reduced interpersonal trust and larger social distance in loneliness. A) High-lonely (HL) participants reported less interpersonal trust. B) Across time points, HL participants stopped at a larger ideal and uncomfortable distance to the experimenter in the stop-distance paradigm. C) Across groups, self-reported interpersonal trust negatively correlated with the mean ideal distance of participants, that is individuals with lower interpersonal trust preferred a greater ideal interpersonal distance. The dashed line represents the 95%-confidence interval of the plotted regression line. All bars represent group means. Error bars indicate standard errors of the mean. Dots on bar plots are jittered for purposes of presentation. *p*-values were calculated using two-sample *t*-tests (A, $n = 82$), mixed analyses of variance (B, $n = 79$), and Spearman's rank correlation (C, $n = 79$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

difference between groups: −7.93 to −0.10; see Figure 1E). Consistent with the notion that loneliness can be perceived by others,[27] the blinded experimenters were significantly better than chance in identifying HL participants after the interaction (78% correct, $\chi^2_{(1)} = 24.82$, $p < 0.0001$; specificity: 72%; sensitivity: 85%). In addition, the experimenters rated HL participants as less trustworthy than LL individuals ($t_{(61.13)} = -2.06$, $p = 0.043$, $d = -0.47$, 95% CI of group difference: −12.82 to −0.20; see Figure 1F).

Collectively, we confirmed that HL participants showed not only a reduced responsiveness to the positive social interaction as evident for self-reported positive affect but also exhibited an attenuated oxytocinergic response. Furthermore, loneliness affected the experimenter's perception of the participants. In the following, we examined the potential impact of interpersonal trust on the impaired social interaction effects in HL participants. For further analyses of the social interaction and immunology, see Supplementary Analyses, Figure S1, Table S2, Supporting Information.

2.2. Loneliness and Reduced Interpersonal Trust

In line with our hypotheses, HL participants reported significantly less interpersonal trust compared to LL individuals ($t_{(80)} = -4.62$, $p < 0.0001$, $d = -1.02$, 95% CI of group difference in scores: −0.79 to −0.31; see **Figure 2**A) and self-reported trust positively correlated with the positive affect after the positive social interaction ($\rho_{(77)} = 0.28$, $p = 0.014$, 95% CI: 0.06 to 0.47). Reduced trust in loneliness was also evident in form of a greater preferred interpersonal distance to strangers. Mixed analyses of variance (ANOVA) with time (before vs after completing the positive social interaction paradigm) as within-subject factor and group (HL vs LL) as between subject factor yielded main effects of group for the ideal ($F_{(1,77)} = 7.17$, $p = 0.009$, $\eta_p^2 = 0.09$, 95% CI of group difference: 0.03 to 0.20 m; see Figure 2B) and slightly uncomfortable distance ($F_{(1,77)} = 4.05$, $p = 0.048$, $\eta_p^2 = 0.05$, 95% CI: 0.001 to 0.13 m). Although distances decreased after the positive interaction (main effect of time for the ideal distance: $F_{(1,77)} = 41.63$, $p < 0.0001$, $\eta_p^2 = 0.35$, 95% CI of decrease: −0.13 to −0.07 m; uncomfortable distance: $F_{(1,77)} = 5.94$, $p = 0.017$, $\eta_p^2 = 0.07$, 95% CI: −0.04 to −0.004 m), the positive interaction was not sufficient to alleviate group differences (all time with group interactions $ps > 0.376$). As expected, self-reported trust negatively correlated with the ideal distance ($\rho_{(77)} = -0.24$, $p = 0.032$, 95% CI: −0.44 to −0.02; see Figure 2C) but not with the distance at which participants felt slightly uncomfortable ($\rho_{(77)} = -0.08$, $p = 0.509$, 95% CI: −0.29 to 0.15).

We further analyzed investment behavior during the trust game by calculating a mixed ANOVA (within-subject factor: game type trust vs risk, between-subject factor: group). The HL subsample was characterized by overall lower investments (main effect of group: $F_{(1,63)} = 4.01$, $p = 0.0495$, $\eta_p^2 = 0.06$; 95% CI of group difference: −2.24 to −0.002 €). Importantly, the absence of significant effects of game type (all $ps > 0.119$ for a main effect or interaction with group) indicates that the implemented risk game constitutes a well-matched control condition for the trust game, as our results show that potential neural differences between conditions cannot be related to different investment choices.

2.3. Loneliness and Trust-Related Brain Activity and Connectivity

To investigate the association of loneliness with trust-related brain activity, we contrasted brain activity during the trust game to the risk game. In a first step, we confirmed that our

implementation of the trust game led to enhanced trust-related brain activity. Whole-brain analyses indeed revealed significantly increased activity during the trust game compared to the risk game in several brain regions associated with trust including the insula, mPFC, hippocampus and amygdala, and TPJ [all $ps < 0.05$ on peak level after family-wise error (FWE) correction; see Supplementary Analyses, Table S3, Supporting Information for details and further whole brain analyses]. We then examined group differences in the reactions to the trust game (trust game > risk game). HL participants showed significantly reduced trust-associated activity in the left AI (−26, 10, −18, $t_{(57)} = 4.07$, FWE-corrected $p = 0.034$; see **Figure 3**A), right NAcc (12, 8, −8, $t_{(57)} = 2.88$, FWE-corrected $p = 0.031$; see Figure 3B), and left amygdala (−20, −8, −16, $t_{(57)} = 3.56$, FWE-corrected $p = 0.042$; see Figure 3C). No significant opposite effects were observed (i.e., increased brain activity during the trust game in HL participants) and groups did not differ in trust-related mPFC or TPJ activity (all FWE-corrected $ps \geq 0.209$). To further characterize the observed interaction of game type and group in the left amygdala, left AI, and right NAcc, we compared parameter estimates using two-sample *t*-tests for each cluster. Results revealed that HL participants did not differ from LL participants in game conditions per se (all $p_{cor} > 0.072$) but rather showed a blunted differentiation (i.e., smaller activity increase) between trust- and risk-related trials in brain regions associated with the evaluation of trustworthiness, risk of betrayal, and reward anticipation.[15]

To probe the robustness of the reduced trust-associated brain activity observed in HL participants, we further analyzed our data by conducting Bayesian inference analyses as implemented in SPM12. Results provide strong evidence that the AI activity is reduced in HL participants compared to LL participants [−26, 10, −18, log odds Bayes factor for attenuated activity in HL participants vs no group differences or enhanced activity in HL participants compared to controls (logBF) = 3.28]. Thus, the Bayes analyses confirmed the results of the frequentist analyses for the left AI, but not for the amygdala or NAcc. Notably, our data also provide strong evidence for reduced mPFC activity that was not detected by the frequentist analyses (0, 52, 10, logBF = 3.62; for further results of the Bayesian analyses that exceed the predefined regions of interest (ROI), see Supplementary Analyses, Figure S2, Supporting Information).

Given that decisions involving trust rely on the interplay between brain regions and neural networks,[15] we explored loneliness-related changes in functional connectivity by calculating generalized psychophysiological interaction (gPPI) analyses. The anatomically defined ROIs were used as seeds in seed-to-voxel analyses and trust-specific connectivity values (i.e., trust game > risk game) were compared between groups. Analyses revealed significant differences in the functional connectivity of the left AI with an occipitoparietal cluster including the cuneus and precuneus between LL and HL participants (−18, −76, 36, $k = 163$, $t_{(57)} = 5.43$, FWE-corrected $p = 0.001$ on cluster level; see **Figure 4**). Specifically, HL participants showed blunted functional connectivity of the left AI with this cluster during the trust game compared to LL participants (post-hoc *t*-test: $t_{(57)} = -3.17$, $p_{cor} = 0.010$, $d = -0.83$, 95% CI of group difference: −0.45 to −0.10), whereas functional connectivity during the risk game did not significantly differ between groups ($t_{(57)} = 1.59$, $p_{cor} = 0.472$, 95% CI of group difference: −0.04 to 0.34). Further post-hoc tests revealed increased functional connectivity during the trust game in LL participants (trust game vs risk game: $t_{(27)} = 3.58$, $p_{cor} = 0.005$, $d = 0.49$, 95% CI of increase: 0.08 to 0.28), while connectivity during the trust game even decreased in HL participants ($t_{(30)} = -4.16$, $p_{cor} = 0.001$, $d = -0.70$, 95% CI decrease: −0.36 to −0.12; for further analyses of connectivity, see Supplementary Analyses, Supporting Information).

Together, these results indicate that reduced interpersonal trust in HL participants might be based on an attenuated recruitment and functional connectivity of limbic regions and, more specifically, the AI during trust decisions. In a next step, we examined whether the observed differences in brain activity and connectivity were in fact associated with interpersonal trust measurements and with the attenuated responsiveness to the positive social interaction in loneliness.

2.4. Brain–Behavior Correlations

Our results confirmed that participants with less self-reported trust also showed less differentiated brain activity (left AI: $\rho_{(57)} = 0.26$, $p = 0.047$, 95% CI: 0.004 to 0.48, see Figure 3A; right NAcc: $\rho_{(57)} = 0.30$, $p = 0.020$, 95% CI: 0.05 to 0.52, see Figure 3B) and that greater trust-related increases in neural activity were associated with higher investments across conditions (left amygdala: $\rho_{(57)} = 0.29$, $p = 0.028$, 95% CI: 0.03 to 0.51, see Figure 3C). Intriguingly, trust-specific connectivity of the left AI with occipitoparietal regions was positively associated with the social interaction-induced increase in positive mood (significant correlations with the increase in positive affect: $\rho_{(56)} = 0.47$, $p = 0.0002$, 95% CI: 0.25 to 0.65, see Figure 4, and positive affect after the task: $\rho_{(56)} = 0.30$, $p = 0.025$, 95% CI: 0.04 to 0.51, but not with baseline positive affect: $\rho_{(56)} = -0.10$, $p = 0.439$, 95% CI: −0.35 to 0.16), indicating that impaired integration of trust-related information relates to diminished benefits of positive social interactions in HL participants. No further significant correlations were observed between neural and behavioral measurements and the oxytocinergic responsiveness to the positive social interaction.

Nevertheless, as the observed brain–behavior correlations might be driven by loneliness, we tested whether the reported correlations were also significant within each group. Analyses confirmed the positive association of AI connectivity with the positive affective responsiveness to the social interaction in LL participants ($\rho_{(26)} = 0.47$, $p = 0.011$, 95% CI: 0.12 to 0.72). This correlation was not significant in HL participants ($\rho_{(28)} = 0.25$, $p = 0.191$, 95% CI: −0.13 to 0.56). Moreover, the correlation of amygdala activity with the monetary investment during the trust and the risk game was found for the HL participants ($\rho_{(29)} = 0.43$, $p = 0.016$, 95% CI: 0.09 to 0.68) but was absent in LL participants ($\rho_{(26)} = 0.15$, $p = 0.458$, 95% CI: −0.24 to 0.49). None of the other reported correlations reached significance within the groups.

2.5. Loneliness, Subclinical Psychiatric Symptoms, and Sex Differences

HL participants were characterized by heightened depressive and anxiety symptoms, childhood maltreatment, and worse sleep quality (all $ps < 0.020$; see Table S4, Supporting Information).

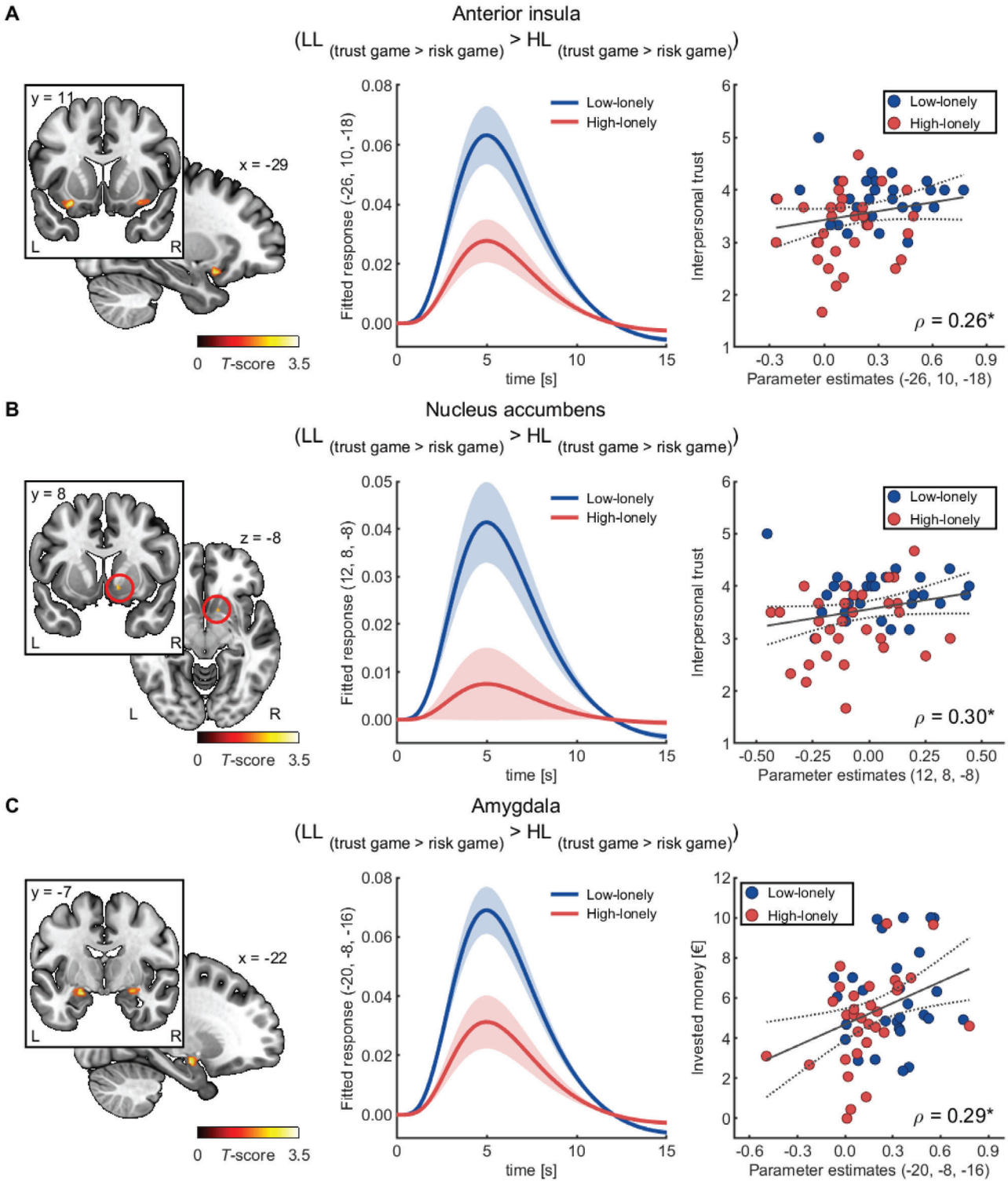

Figure 3. Reduced trust-associated brain activity in high-lonely (HL) participants. A) HL participants exhibited less activity in the trust game relative to the risk game in the left anterior insula, B) the right nucleus accumbens, and C) the left amygdala. In the pooled sample, responses in the anterior insula and the right nucleus accumbens positively correlated with self-reported trust, while parameter estimates of the left amygdala activity during the trust game (compared to the risk game) were positively associated with the invested money across conditions. For illustration purpose clusters are shown with significance levels of $p < 0.05$ uncorrected. The shaded areas show the standard error of the mean of the estimated time courses based on the canonical hemodynamic response function as used in SPM12 multiplied by the parameter estimates of the trust game > risk game contrast. The dashed lines represent the 95%-confidence intervals of the plotted regression lines. Abbreviations: L, left; LL, low-lonely; R, right. *p*-values were calculated using Spearman's rank correlations ($n = 59$). * $p < 0.05$.

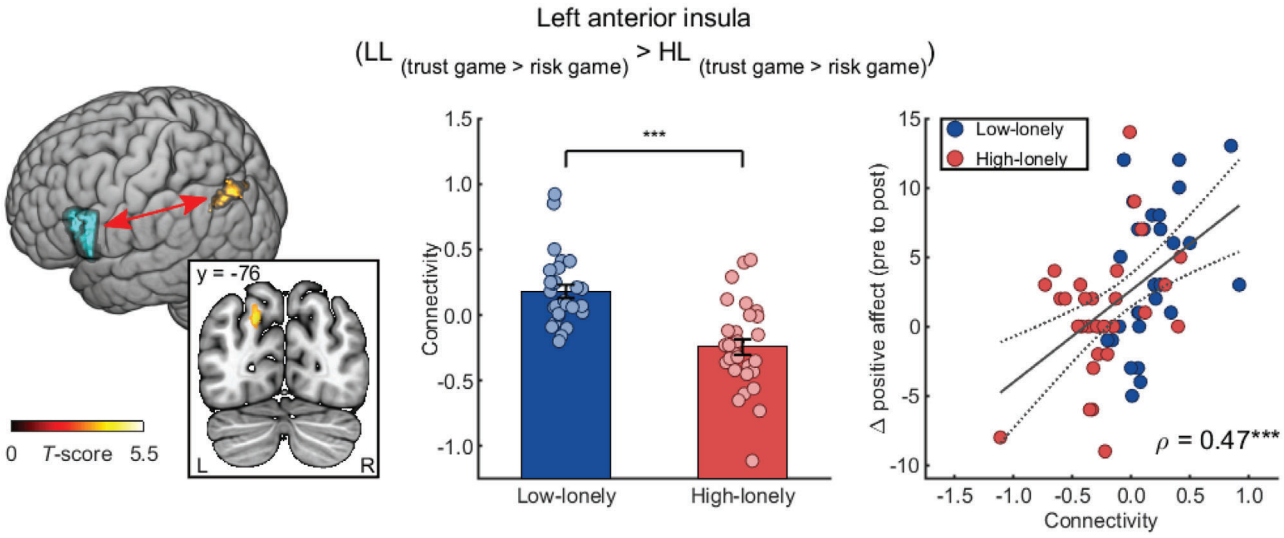

Figure 4. Reduced trust-associated connectivity of the anterior insula in high-lonely (HL) participants. HL participants showed altered trust-associated connectivity of the left anterior insula (blue sphere) with an occipitoparietal cluster including the cuneus and the precuneus. This connectivity of the left anterior insula during the trust game (compared to the risk game) positively correlated with the interaction-induced changes in positive affect across groups. The dashed line represents the 95%-confidence interval of the plotted regression line. All bars represent group means. Error bars indicate standard errors of the mean. Dots on bar plots are jittered for purposes of presentation. Abbreviations: L, left; LL, low-lonely; R, right. *p*-values were calculated using two-sample *t*-tests ($n = 59$) and Spearman's rank correlation ($n = 58$). *** $p < 0.001$.

Furthermore, HL participants reported smaller and less diverse social networks (all *p*s < 0.002; see Table S4, Supporting Information). To assess whether the observed associations of loneliness with behavioral, neuroendocrine, and neural alterations are mediated by these psychiatric characteristics of HL participants, we conducted mediation analyses with depressive and anxiety symptoms and childhood maltreatment scores as mediator variables and group as independent variable. None of the reported group effects was confounded by any of the tested mediators (all 95% CIs of mediation effects overlapped with zero) except for the reduced AUC_I in salivary oxytocin levels after completion of the positive social interaction task which was mediated by depressive symptomatology ($\beta = 0.22$, SE = 0.11, 95% CI: 0.04 to 0.47).

Notably, the sex of the participants did not significantly influence the strength of the reported associations of loneliness with brain activity or connectivity: no interactions of group with sex were observed for trust-associated AI activity (group-by-sex interaction: $B = 0.12$, $t_{(55)} = 1.04$, $p = 0.302$, 95% CI: −0.11 to 0.35) or connectivity ($B = -0.11$, $t_{(55)} = -0.69$, $p = 0.496$, 95% CI: −0.42 to 0.21) or for trust-associated amygdala ($B = -0.02$, $t_{(55)} = -0.19$, $p = 0.847$, 95% CI: −0.25 to 0.21), NAcc ($B = -0.16$, $t_{(55)} = -1.53$, $p = 0.131$, 95% CI: −0.37 to 0.05), or mPFC activity ($B = 0.20$, $t_{(55)} = 0.65$, $p = 0.522$, 95% CI: −0.42 to 0.83). Likewise, the sex of the participants did not significantly influence other findings, although the association between loneliness and general trust appears to be more pronounced in men than women (group-by-sex interaction: $B = -0.43$, $t_{(78)} = -1.82$, $p = 0.073$, 95% CI: −0.90 to 0.04; group effect in female participants: $B = -0.34$, $p = 0.048$, 95% CI = −0.67 to −0.002; male participants: $B = -0.77$, $p < 0.001$, 95% CI = −1.10 to −0.43; all further interactions of group with sex: *p*s > 0.130). For further moderation analyses, see Supplementary Analyses, Supporting Information.

3. Discussion

Our study sought to investigate a trust-based mechanism underlying the attenuated reactivity to positive social interactions in a pre-stratified sample of HL and LL participants. As hypothesized, HL individuals exhibited reduced affective responses to the positive social interaction and reported less interpersonal trust. Moreover, during initial trust decisions, blunted AI activity in HL participants was consistently found across frequentist and Bayesian analyses and was accompanied by reduced functional connectivity of the AI to an occipitoparietal cluster, including the cuneus and precuneus, which correlated with attenuated affective reactivity to the positive social interaction. Frequentist analyses further indicate diminished trust-associated brain activity in the amygdala and NAcc, while Bayesian analyses provide strong evidence for blunted mPFC activity in HL participants. Further explorative analyses revealed attenuated oxytocinergic responsiveness to the positive discussion in HL participants and that HL participants were rated to be less trustworthy by the unfamiliar experimenter. Notably, although the HL sample was characterized by heightened psychiatric symptomatology, neither depression or social anxiety scores nor reported childhood maltreatment mediated the observed neural group differences.

Our results confirmed the findings of previous studies reporting reduced responsiveness to positive social interactions in lonely individuals:[12,13] while the LL sample showed the expected increase in positive affect and salivary oxytocin concentrations, these responses were significantly diminished in HL participants. Furthermore, consistent with previous observations,[28] HL participants preferred a greater interpersonal distance. Although this greater interpersonal distance might also reflect safety behavior due to the weakened immune system in lonely

individuals[10] (also see Supporting Information), our results strongly support theoretical framework suggesting that negative biases have a detrimental effect on social interactions in loneliness,[11] as impaired interpersonal trust significantly correlated with a preference for larger interpersonal distance and reduced positive mood after the positive social interaction.

Mechanistically, our findings might indicate that impaired trust evaluations could be rooted in attenuated limbic reactivity. Our observation of reduced trust-associated activation in the AI is consistent with recent studies highlighting the AI as a key region contributing to trust decisions specifically during the single-round trust game.[29] The AI encodes the trustworthiness of faces[30] and is fundamentally involved in integrating interoceptive information from limbic regions including the amygdala and the NAcc. Interestingly, the AI initializes the processing of salient information in the prefrontal cortex, encodes the incentive value of stimuli,[31] and changes in the glucose metabolism of the AI positively correlate with changes in social interest.[32] Thus, the reduced trust-related AI activity might indicate a compromised integration of amygdalar and striatal trust signals that might contribute to the overall nihilistic feeling that nobody can be trusted. Moreover, HL participants showed an altered interplay of the AI with a brain cluster including parts of the precuneus. The precuneus is a central hub of the default mode network and contributes to self-referential operations including self-consciousness and the mental representation of the self.[33] Importantly, the functional connectivity of the precuneus with the AI during rest has been previously found to predict trust and reciprocity in non-lonely individuals.[34,35] During positive social interactions, the precuneus might contribute to the continuously updated representation of a positive self-image that could reinforce the reward value of social interactions.[36] In fact, connectivity of the AI with the precuneus correlated with the beneficial effects of our positive social interaction.

Furthermore, our results indicate a diminished recruitment of the amygdala, the NAcc, and the mPFC during trust-processing in lonely individuals. The mPFC has been previously implicated in loneliness,[14,37] but our findings have to be interpreted with caution as the reduced trust-associated activity in HL participants could not be replicated across different analytic approaches. Like the AI, the mPFC is known to interact with various limbic regions, encode the expected value of stimuli,[38,39] evaluate trait characteristics of others,[40] and predict trusting behavior.[34,41] The observed attenuated mPFC activity during the trust game might thus reflect a reduced utility of social stimuli, as lonely individuals potentially prefer safety behavior irrespective of the trustworthiness of the partner.[42] In addition, the reduced mPFC activity might be linked to the attenuated recruitment of the amygdala and the NAcc.[38,42]

The amygdala is crucially involved in the processing of social information, such as the trustworthiness or ambiguity of social stimuli, and previous lesion studies provide strong evidence that an intact amygdala is necessary for developing appropriate interpersonal trust.[30,43] Notably, like the AI, the amygdala encodes not only the negative valence of stimuli but also signals highly untrustworthy and trustworthy faces.[30] Together with the association of reduced amygdala reactivity and lower monetary investment across conditions, our results could indicate that HL individuals might be less able to reliably evaluate the trustworthiness of strangers. This way, reduced amygdala sensitivity for trustworthiness evaluations might be a reinforcing mechanism for a default distrust mode as safety behavior in loneliness.

Moreover, intact amygdala projections to the NAcc are important to guide action selection in situations involving reward uncertainty[44] and the NAcc showed diminished activity during trust decisions in HL individuals. The NAcc consistently responds to trust decisions during the multi-round trust game,[29] but since we implemented a single-round version of the trust game, the striatal hypoactivation might reflect a general attitude of reduced trust toward strangers rather than previous learning experiences with the individual trustees. Nevertheless, our results might also indicate a reduced reward value of social stimuli in loneliness per se[12] irrespective of the expected outcome during the trust game.

Notably, HL participants did not differ from the LL sample in trust-related activity of the TPJ, known to play a crucial role in inferring the mental state and temporary goals of other persons.[17,45] As such, our findings point to a compromised integration of interoceptive trust signals and mental self-representation mediated by the functional interplay between the AI and precuneus as well as an impaired processing of trustworthiness and stimulus utility in the amygdala, NAcc, and mPFC, rather than altered inferences about the mental states of others as primarily processed in the TPJ.

Of note, diminished reactivity to social interactions in HL individuals was not limited to self-reported mood but also evident in significantly lowered endogenous oxytocin responsiveness. Oxytocin is crucially involved in human affiliation and trust[23–25] and recent studies have highlighted the potential of intranasally administered oxytocin to increase interpersonal trust behavior in participants with a low disposition to trust.[46,47] We have previously found that social synchrony, that is the temporal coordination of social behavior and physiological processes among individuals, evokes heightened endogenous oxytocin release, which predicts interactive reciprocity[48] and that intranasal administration of oxytocin increases synchrony during dance.[49] Social synchrony is essential for human bonding and has been associated with positive affect and prosocial behavior.[50] While the electrodermal and heart rate measurements demonstrate a normal arousal response to the positive social interaction, HL individuals not only reported less interpersonal trust but they were also rated as less trustworthy and the blinded experimenter was able to recognize them better than by chance. Thus, our findings support previous reports about the social transmission of loneliness[27] and suggest that the impaired trust evaluation may hamper social synchrony, which in turn can explain the lower perceived trustworthiness of HL individuals.[51] Along these lines, dysfunctional social interactions in loneliness may result from a reciprocally-reinforced bias in trust behavior.

The current study has several limitations. The cross-sectional design of the current study does not allow causal inferences about the relationship between interpersonal trust, loneliness, and the beneficial effects of positive social interactions. Although preliminary evidence supports the notion of reduced interpersonal trust as a risk factor for rather than a consequence of loneliness,[11] future longitudinal studies are required to directly test the causality of this model. Likewise, experimental studies using neurofeedback training, human lesion models, or transient lesions via

non-invasive brain stimulation are needed to prove the causal involvement of the observed trust-associated neurocircuit in loneliness. Furthermore, interpersonal synchrony needs to be characterized in naturalistic settings with two HL participants and mixed dyads of HL and LL individuals. Notably, although moderation analyses did not reveal significant interactions of loneliness with the participants' sex, this does not exclude the possibility of sex differences in other loneliness-related domains or population-based measurements. For instance, previous studies found sex-specific associations of loneliness with brain structure and resting state functional connectivity using the UK biobank population.[14,52,53]

4. Conclusion

Collectively, our results indicate compromised integration of trust-related information as a potential reciprocally-reinforced mechanism that might contribute to dysfunctional social interactions in loneliness, thereby reducing the motivation to reconnect and promoting avoidance behavior. Neurobiologically-informed interventions with cognitive bias modification procedures should target the self-reinforcing loop of distrust to improve the beneficial reactivity to positive social interactions and alleviate the debilitating health consequences of perceived social isolation.

5. Experimental Section

Participants and Study Design: To investigate the impact of current loneliness on interpersonal trust, the reactivity to positive human interactions, and its underlying neurobiological mechanisms, a quasi-experimental design with a sample of pre-stratified healthy volunteers scoring high (≥50, i.e., at least one standard deviation above the mean score of students, cf. ref. [54]) or low (≤25, i.e., at least one standard deviation below the mean) on the revised UCLA Loneliness Scale (UCLA-L) was used.[54] For recruitment, an online survey assessing the UCLA-L score was disseminated by means of online advertisement and public postings. A total of 410 participants out of 3678 subjects who filled out the UCLA-L scale met the inclusion criteria (see Supplementary Methods, Supporting Information) and out of these 410 participants, 91 subjects agreed to participate and were invited to a screening session. Nine participants were excluded after the screening session since they were not eligible for enrolment, resulting in a final sample of 42 HL (female $n = 21$) and 40 LL participants (female $n = 20$) in accordance with the planned sample size of 80 participants (for details of the a-priori power analysis, see Supplementary Methods, Supporting Information). Groups were matched for age (HL mean age ± SD: 26.55 ± 6.80 years, LL: 27.13 ± 8.18 years; $t_{(80)} = 0.35$, $p > 0.05$, 95% CI of group difference: −3.88 to 2.72 years) and sex and did not differ regarding sociodemographic factors (all ps > 0.05; see Table S1, Supporting Information). All participants provided written informed consent and received monetary compensation for participation. The study was approved by the local ethics committee of the Medical Faculty of the University of Bonn, Germany (study number 016/18), and carried out in accordance with the latest revision of the Declaration of Helsinki. Data analysis was preregistered prior to conducting any analyses (https://osf.io/x47ke; results regarding the preregistered hypothesis #3 will be published elsewhere).

Psychological Variables: Participants completed questionnaires measuring interpersonal trust, the social network size and diversity, and sleep quality. As loneliness is often associated with psychiatric symptomatology, depressive symptoms, social anxiety, and childhood maltreatment were also assessed. For details, see Supplementary Methods, Supporting Information.

Trust Game: An adapted version of an established trust game[21] was implemented. Briefly, two players, the investor and the trustee, started each round with an endowment of 10 €. The investor chose the amount of money he/she wanted to invest in an unknown trustee. The invested money was tripled and added to the trustee's account. The trustee could keep all of the money for him/herself or share the money with the investor so that both players ended with the same amount of money (10 € plus the invested amount). Decisions of the participants in the role of the trustee were collected for all possible investments during the screening session (see also Supplementary Analyses, Supporting Information). Participants were informed that they would play the trust game in the role of the investor against other participants of the study (as trustees) and that their own payment depended on a randomly chosen trial (100% of the final endowment after consideration of the trustee's decision was paid).

During fMRI, participants then played the trust game as investor without receiving feedback about the pre-recorded decisions of the trustees to explore the impact of loneliness on rapid trustworthiness decisions during initial encounters. In a control condition, participants played a risk game in which they invested money in a computer (which would randomly decide whether the money would be shared).

As choice options and possible outcomes were exactly the same during the trust game and the risk game, the conditions differed only with respect to the social risk of betrayal when playing with a human counterpart. Thus, when analyzing trust-related decisions and associated brain activity, it was crucial to validate whether participants believed they were playing against real persons as no differences should be observed otherwise. Participants were therefore asked both verbally and via questionnaire whether they believed the instructions. For details, see Supplementary Methods, Supporting Information.

Positive Social Interaction Paradigm: After completion of the fMRI scan, participants moved to the testing room, which was prepared for the positive social interaction paradigm. The task consisted of a semi-structured 10-min conversation between the participant and a same-sex unfamiliar experimenter. Participants were told to talk about 1) plans for a fictive lottery win, 2) positive childhood memories, and 3) hobbies and interests. High-quality photographs presenting examples for activities (e.g., traveling around the world or buying a sports car) were used to facilitate the start of the conversation. Participants and the experimenter tried to find similarities in the discussed topics. Importantly, the experimenter was blinded regarding the group assignment of the participant (HL vs LL) and unknown to the participants prior to the fMRI session in all cases. Participants self-reported mood and affect before and after completing the positive social interaction paradigm (see Supplementary Methods, Supporting Information). After finishing the task, both the participant and experimenter rated the valence of the discussion as well as trustworthiness and likeability of each other using VAS. The experimenter further estimated the experimental group of the participant (HL vs LL) to examine whether loneliness might be detected by others after the positive interaction.

Baseline EDA and an electrocardiogram were collected for 5 min prior to the positive social interaction paradigm and throughout the entire social interaction. Finally, saliva samples were collected before, immediately after the social interaction paradigm, and 15 min after completion of the positive social interaction task to obtain salivary oxytocin, cortisol, and IgA levels in addition to baseline immune parameters and oxytocin levels in blood (see Supplementary Methods, Supporting Information).

Interpersonal Distance Paradigm: The interpersonal distance as an indirect index of trust toward strangers was measured by an adapted version of an established stop-distance paradigm.[22] Participants moved toward an unfamiliar experimenter (the same experimenter who conducted the positive social interaction) from a start distance of 2 m and stopped at their ideal distance. In a second trial, participants were instructed to stop at a distance at which they felt slightly uncomfortable. The start and final chin-to-chin distance were measured with a digital laser measurer (error: ± 0.003 m). Both conditions were measured before and after the positive social interaction.

fMRI Data Analysis: fMRI data were acquired with a 3T Siemens TRIO MRI system (Siemens AG, Erlangen, Germany) using a T2*-weighted echoplanar (EPI) sequence and preprocessed and analyzed using

standard procedures in SPM12 (Wellcome Trust Center for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab (The MathWorks Inc., Natick, MA; see Supplementary Methods, Supporting Information). A two-stage approach based on the general linear model implemented in SPM12 was used for statistical analyses. On the first level, participants' individual data were modeled using a fixed-effects model. Onsets and durations of the experimental conditions were modeled by a stick function convolved with a hemodynamic response function (HRF). Movement parameters were included in the design matrix as confounds. In line with previous research investigating trust[21] and according to the hypothesis of altered trust-associated brain activity in HL participants, individual brain activity was contrasted during the trust game with the risk game (trust game > risk game) as main contrast of interest on the first level. Groups were compared by using two-sample *t*-tests on the second level ($HL_{\text{trust game > risk game}} > LL_{\text{trust game > risk game}}$, $LL_{\text{trust game > risk game}} > HL_{\text{trust game > risk game}}$; for further details and analyses, see Supplementary Methods, Supporting Information). The main analyses of fMRI data focused on independently defined brain regions (ROIs) known to be involved in motivational, affective, and cognitive processes during the trust game consisting of the bilateral amygdala, AI, mPFC, and TPJ[15] (see Supplementary Methods, Figure S3, Supporting Information). The NAcc was further included as ROI associated with reward anticipation during the trust decision stage because the NAcc was found to show altered activity as a function of loneliness.[12] *p* values smaller than 0.05 after FWE-correction based on the size of the ROI (i.e., small volume correction) were considered significant. Whole-brain analyses were calculated across groups for task validation. Parameter estimates of significant contrasts were extracted using marsbar (http://marsbar.sourceforge.net) and further analyzed to disentangle interactions by calculating Bonferroni-corrected post-hoc *t*-tests. To account for the liberal threshold of small volume corrections in the ROI analyses, the robustness of observed group differences in trust-associated brain activity was probed by conducting Bayesian inference analyses as implemented in SPM.[55] Results were thresholded with the following criteria: a log odds Bayes factor threshold of log BF ≥ 3 (strong evidence,[56]) for at least small group effects (i.e., an effect size threshold of 0.2).

A gPPI analysis was conducted using the CONN toolbox 19.b (www.nitrc.org/projects/conn, RRID:SCR_009550) and the same statistical model as outlined above (for details, see Supplementary Methods, Supporting Information). Those ROIs that showed significant effects during the fMRI trust game (i.e., left amygdala, left AI, right NAcc) were used as seed regions in planned seed-to-voxel analyses, while all other ROIs were used as seed regions in additional exploratory seed-to-voxel analyses (see Supplementary Analyses, Supporting Information). For each participant, interaction terms of the psychological factor (effects of task conditions convolved with a canonical HRF) and the physiological factor (seed ROI BOLD time series) were computed on the first level. Bivariate regression measures were used to provide the relative measure of connectivity compared to the implicit baseline (defined by the zero values of the interaction term). On the second level, trust-specific connectivity values between groups were compared using 2 × 2 mixed ANOVA interactions ($HL_{\text{trust game > risk game}} > LL_{\text{trust game > risk game}}$; $LL_{\text{trust game > risk game}} > HL_{\text{trust game > risk game}}$) to test the hypothesis of altered connectivity in loneliness. Results were thresholded at an FWE-corrected *p*-value < 0.05 after an initial cluster-forming height threshold of $p < 0.001$. Beta weights of significant effects of interest were extracted and further analyzed by calculating Bonferroni-corrected post-hoc *t*-tests.

Behavioral and Questionnaire Data Analysis: Statistical analyses were performed using SPSS 24 (IBM Corp., Armonk, NY). Questionnaire data were compared between groups using two-sample *t*-tests and chi-square tests. All behavioral data were analyzed using mixed-design ANOVAs and Bonferroni-corrected post-hoc *t*-tests. If the assumption of sphericity was significantly violated as assessed by Mauchly's tests, Greenhouse–Geisser corrections were applied. The sociality condition of the trust game served as within-subject factor (trust game vs risk game), while group constituted the between-subject factor (HL vs LL). The hypothesized group differences in the response to the positive social interaction paradigm (self-reported affect and mood) and the interpersonal distance task (separated for comfortable and uncomfortable distance) were analyzed with time (before vs after social interaction) as within-subject factor and group as between-subject factor. Analyses of the trust game excluded participants who did not believe the instructions as stated verbally or during the exit questionnaire ($n = 8$ HL, $n = 9$ LL). For analyses of the positive social interaction paradigm, participants who were not fluent in German were excluded ($n = 3$ HL). Chi-square tests were used to calculate whether the estimation of the experimental group (HL vs LL) by the experimenter differed significantly from chance.

Psychophysiology and Neuroendocrinology Analysis: The SCL and heart rate (BPM) were analyzed using mixed ANOVAs including the within-subject factor time (baseline vs social interaction) and the between-subject factor group (HL vs LL). The difference between the duration of the baseline acquisition and the duration of the social interaction task was included as covariate to control for changes in psychophysiology related to differences in data acquisition times.

Baseline differences in the salivary and plasma oxytocin levels, salivary cortisol and IgA concentrations, and in blood parameters (serum C-reactive protein, interleukin-6, 25-hydroxyvitamin D, oxytocin, and cell count parameters) were compared between groups using two-sample *t*-tests. The AUC_I (see Supplementary Methods, Supporting Information) was calculated for salivary oxytocin, cortisol, and IgA levels, tested against zero to examine the responsiveness to the positive social interaction across groups, and compared between groups, again using two-sample *t*-tests.

Correlation, Mediation, and Moderation Analyses: To examine the hypothesis that altered brain activity and connectivity in HL participants relate to the observed behavioral group differences, parameter estimates of trust-specific brain activity and connectivity were correlated with the behavioral variables that were associated with loneliness (for details, see Supplementary Methods, Supporting Information). To further explore the relationship of interpersonal trust with behavioral data, the self-reported interpersonal trust was also correlated with those variables. Furthermore, correlation analyses were calculated separately for each group.

To examine whether observed group effects (main effects of group or interactions with group) might be driven by psychiatric symptomatology, mediation analyses were calculated and tested for indirect effects of loneliness via psychiatric symptomatology. Thus, it was examined whether the observed effects of loneliness might be partially or fully based on the psychiatric symptoms associated with loneliness.

In addition, to expand the understanding of the interplay of loneliness and psychiatric symptomatology, moderation analyses were conducted to investigate potential interaction effects. This way, it was tested whether psychiatric symptomatology might potentiate observed effects associated with loneliness (i.e., stronger effect of loneliness in participants with higher psychiatric symptoms) or reduce the impact of loneliness (i.e., less effect of loneliness in participants with higher psychiatric symptoms). Likewise, moderation analyses were conducted with the sex of the participants as moderator variable to examine whether the effects of loneliness differed between sexes. For details, see Supplementary Methods, Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

J.L. and D.S. designed the experiment; J.L., T.E., and E.K. conducted the experiments; J.L., E.K., and D.S. analyzed the data. All authors wrote the manuscript. All authors read and approved the manuscript in its current version.

Data Availability Statement

The behavioral data that support the findings of the current study are openly available in the repository of the Open Science Foundation at https://osf.io/p6jxk/. The unthresholded statistical maps of the fMRI results can be accessed at https://neurovault.org/collections/BMDUCOHK/.

Keywords

interpersonal trust, loneliness, oxytocin, social brain, social interaction

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3.3. Publication 3: Behavioral and neural dissociation of social anxiety and loneliness

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Behavioral/Cognitive

Behavioral and Neural Dissociation of Social Anxiety and Loneliness

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Loneliness is a public health concern with detrimental effects on physical and mental well-being. Given phenotypical overlaps between loneliness and social anxiety (SA), cognitive-behavioral interventions targeting SA might be adopted to reduce loneliness. However, whether SA and loneliness share the same underlying neurocognitive mechanisms is still an elusive question. The current study aimed at investigating to what extent known behavioral and neural correlates of social avoidance in SA are evident in loneliness. We used a prestratified approach involving 42 (21 females) participants with high loneliness (HL) and 40 (20 females) participants with low loneliness (LL) scores. During fMRI, participants completed a social gambling task to measure the subjective value of engaging in social situations and responses to social feedback. Univariate and multivariate analyses of behavioral and neural data replicated known task effects. However, although HL participants showed increased SA, loneliness was associated with a response pattern clearly distinct from SA. Specifically, contrary to expectations based on SA differences, Bayesian analyses revealed moderate evidence for equal subjective values of engaging in social situations and comparable amygdala responses to social decision-making and striatal responses to positive social feedback in both groups. Moreover, while explorative analyses revealed reduced pleasantness ratings, increased striatal activity, and decreased striatal-hippocampal connectivity in response to negative computer feedback in HL participants, these effects were diminished for negative social feedback. Our findings suggest that, unlike SA, loneliness is not associated with withdrawal from social interactions. Thus, established interventions for SA should be adjusted when targeting loneliness.

Key words: amygdala; fMRI; loneliness; social anxiety; striatum

Significance Statement

Loneliness can cause serious health problems. Adapting well-established cognitive-behavioral therapies targeting social anxiety might be promising to reduce chronic loneliness given a close link between both constructs. However, a better understanding of behavioral and neurobiological factors associated with loneliness is needed to identify which specific mechanisms of social anxiety are shared by lonely individuals. We found that lonely individuals show a consistently distinct pattern of behavioral and neural responsiveness to social decision-making and social feedback compared with previous findings for social anxiety. Our results indicate that loneliness is associated with a biased emotional reactivity to negative events rather than social avoidance. Our findings thus emphasize the distinctiveness of loneliness from social anxiety and the need for adjusted psychotherapeutic protocols.

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Introduction

Loneliness is a painful condition with detrimental effects on mental and physical health (Quadt et al., 2020). As such, loneliness has been identified as a risk factor for premature mortality comparable with smoking or obesity (Holt-Lunstad et al., 2010). Consequently, loneliness has come into focus of politics and clinicians as a major public health concern with high economic costs for society (Jeste et al., 2020; Mihalopoulos et al., 2020). With social distancing measures in most countries around the world, COVID-19 is expected to have vast impact on physical and mental health, particularly in people inflicted by poor

resilience to social adversity because of preexisting low levels of social integration (Galea et al., 2020; Vindegaard and Benros, 2020), emphasizing the urgent need of interventions to target loneliness. Adjusting established cognitive-behavioral therapies targeting-related psychopathology, such as depression or social anxiety (SA) (Heinrich and Gullone, 2006), seems promising to accelerate the development of treatments to reduce loneliness. However, previous studies indicated that loneliness and depression are distinct constructs based on unique neurobiological mechanisms (Cacioppo et al., 2010; Shao et al., 2019). Conversely, it is still unclear whether loneliness shares neurobiological substrates with SA, which would allow rapid co-optations of psychotherapeutic protocols.

Recent findings highlight close links between loneliness and SA symptoms (Bruce et al., 2019; Maes et al., 2019) and identified SA as predictor for future loneliness (Lim et al., 2016; Danneel et al., 2019). For instance, SA was found to be consistently associated with social isolation, lower perceived social support, and poor friendship quality, resulting in decreased relationship satisfaction, which is a key feature of loneliness (Peplau and Caldwell, 1978; Teo et al., 2013; Porter and Chambless, 2014; Rapee et al., 2015; Rodebaugh et al., 2015). Likewise, the avoidance of social situations is known to be a core mechanism of SA; and although loneliness might have evolved as a motivation to reconnect with others, social avoidance is also hypothesized to be preferred by lonely individuals (Cacioppo and Cacioppo, 2018).

Existing SA intervention programs are often based on cognitive models of SA (Clark and Wells, 1995), which posit an exaggerated fear of evaluation as a core etiologic mechanism of psychopathology. Indeed, current neurocircuitry models of SA disorder emphasize amygdala hyperreactivity to social stimuli (Etkin and Wager, 2007; Bruhl et al., 2014). Conversely, the neural responsiveness to social rewards seems to be reduced in individuals with SA (Richey et al., 2017; Schultz et al., 2019), potentially resulting in reduced positive affect in response to social interactions (Kashdan and Collins, 2010). Similarly, lonely individuals exhibit attenuated responsiveness to positive social interactions (Lieberz et al., 2021), and preliminary evidence indicates that alterations in amygdala structure and function are associated with loneliness (for a comprehensive review of neurobiological correlates of loneliness, see Lam et al., 2021; Morr et al., 2022).

The current study therefore aims at examining whether mechanisms underlying SA could also underlie loneliness. We recruited a prestratified sample of 42 healthy participants with high (high-lonely [HL]) and 40 participants with low (low-lonely [LL]) loneliness scores. During fMRI, the participants completed a social gambling task as used by Schultz et al. (2019) to measure the behavioral and neural responsiveness to social decision-making and social feedback. Given the intertwined phenotype of SA and loneliness, we hypothesized that lonely individuals would show increased SA symptomatology and in turn behavioral and neural response patterns associated with social avoidance (compare Schultz et al., 2019). Specifically, we hypothesized decreased subjective values of engaging in social situations, increased amygdala activation during social decision-making and social feedback, and decreased reward-associated responses of the nucleus accumbens (NAcc) to positive social feedback in lonely participants. Moreover, we explored distinct behavioral and neural response patterns in loneliness that have not been previously found to be associated with SA (i.e., responsiveness to negative social feedback). We controlled for the influence of SA and further potential confounding variables for all observed correlates of loneliness.

Materials and Methods

Participants. We recruited a sample of 82 (of a stratified sample of 3678 adults; 41 females, mean age ± SD: 26.83 ± 7.47 years) (see Lieberz et al., 2021) prestratified healthy HL ($n = 42$) and LL volunteers ($n = 40$) as assessed by the revised version of the UCLA loneliness scale (UCLA-L) (Russell et al., 1980). HL Participants were characterized by UCLA-L scores of ≥50 (i.e., at least 1 SD above the mean score of students) (compare Russell et al., 1980), whereas LL participants were characterized by scores of ≤25 (i.e., at least 1 SD below the mean score of young adults). All participants fulfilled the following inclusion criteria: aged 18-65, no current physical or psychiatric disorder as assessed via self-disclosure and by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998), no psychotherapy, no current psychotropic medication, no illicit drug use in the previous 4 weeks, right-handed, and eligibility for MRI scanning. The sample size was based on an *a priori* power analysis (compare Lieberz et al., 2021). The analysis using G*Power 3 (Faul et al., 2007) indicated that at least 71 participants were needed to reliably replicate a previously reported loneliness effect on ventral striatum/amygdala activity (Cacioppo et al., 2009) with a power of 0.99 ($\alpha = 0.05$). To account for possible missing data and dropouts, we planned to test at least 80 participants, resulting in the final sample size of 82 participants. For a comprehensive description of the prestratification approach, see Lieberz et al. (2021).

All participants gave written informed consent. The study was approved by the institutional review board of the Medical Faculty of the University of Bonn (study number 016/18) and conducted in accordance with the latest revision of the Declaration of Helsinki.

Experimental design and statistical analyses. Following the screening of inclusion criteria, participants completed a virtual auction task to measure the individual monetary value associated with receiving positive or avoiding negative social feedback. To further measure the participants' subjective value of engaging in social situations, participants competed a social gambling task (compare Schultz et al., 2019) during a separate test session and repeated the task during fMRI on the same day. Data collection was completed before the start of the COVID-19 pandemic. The analysis plan was preregistered before conducting any analyses (https://osf.io/x47ke). All data used in this study are openly available (https://osf.io/p6jxk/ and https://neurovault.org/collections/VNYRMORR/).

Social gambling task. Each trial of the social gambling task consisted of a decision and a feedback stage (Fig. 1). During the decision phase, participants could choose a risky (a dice game with a virtual human or computer partner with equiprobable outcomes of 3 or 0 €) or a safe option (a fixed payoff ranging from 0 to 3 € in steps of 50 cents) with no imposed time limit. Human partners were indicated by the name and picture of 1 of 4 partners, while the computer control condition was indicated by a picture of a computer. If participants chose the risky option, either a positive or a negative feedback video of the partner (human or computer) was shown (feedback phase), depending on the outcome of the trial (win or loss). As such, the human feedback video displayed the virtual human partner expressing either admiration or condescension. All human pictures and videos were taken from a validated database (Kaulard et al., 2012). In the computer control condition, the feedback was given by a video of a checkmark (participant won) or a cross (participant lost). Each feedback video was presented 2 times in immediate succession. If participants chose the safe option, a sentence confirmed the payoff. Each human partner was paired twice with each possible amount of money offered as alternative for the risky option, resulting in 56 trials. Likewise, participants completed 56 trials of the control condition. After finishing the task, participants rated the pleasantness of each positive and negative feedback video on a visual analog scale ranging from 0 ("not pleasant at all") to 100 ("very pleasant"). Moreover, for each participant, individual certainty equivalents of the risky option (termed CE50, i.e., the certain payoff for which a participant would be indifferent between the risky and safe options: they would

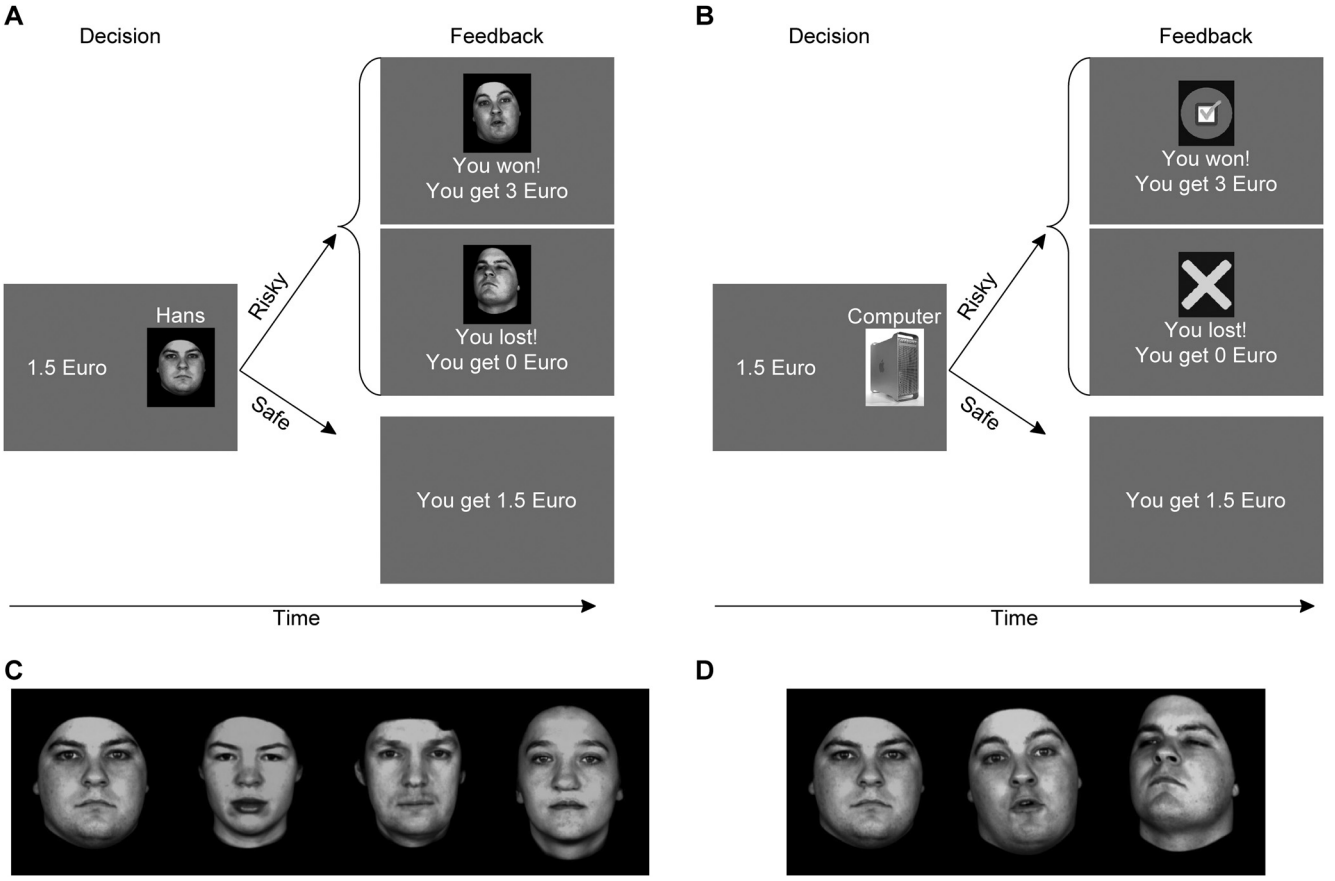

Figure 1. Social gambling task. The social gambling task included a human (***A***) and a computer (***B***) condition, and each trial consisted of a decision and a feedback stage. During the decision phase, participants could choose a risky or a safe option (a uniformly distributed random fixed payoff ranging from 0 to 3 € in steps of 50 cents). If participants chose the risky option and won the trial, a positive feedback video of the partner was shown and the participant got 3 €. If participants lost the trial, they received no payoff and a negative feedback video was presented. The human feedback video displayed the virtual human partner expressing either admiration (participants won) or condescension (participant lost). In the computer control condition, the feedback was given by a video of a checkmark (participant won) or a cross (participant lost). If participants chose the safe option, a sentence confirmed the payoff. During fMRI, the partner was indicated by the name of the virtual human partner or the word "computer" only. ***C***, The four virtual human partners with neutral facial expression. ***D***, One of the partners with neutral, admiring, and condescending facial expressions (left to right). The admiring and condescending expressions were presented as videos during the feedback stage. See also Schultz et al. (2019, their Fig. 1).

choose each option with equal probability) were estimated separately for the computer and the human partners by fitting participants' choices as a function of the difference between the expected values of the safe and risky options with a cumulative Gaussian function. CE20 and CE80 (i.e., certain payoffs associated with choosing the safe option with 20% and 80%, respectively, probability) were similarly estimated. The subjective value of engaging in social situations was defined as the individual difference between the estimated CE50 for human partners compared with the computer partner.

The task was repeated during fMRI with the following adjustments: the partner for each trial (1 of 4 human partners or the computer) was chosen randomly and indicated by the name of the partner (no face was shown at this stage) or the word "computer." Furthermore, the fixed payoff offered in the safe option varied randomly between the three individually determined values CE20, CE50, and CE80. Using individualized payoffs as a safe alternative enabled us to equate the number of risky and safe choices across participants. Participants responded with their index fingers using an MRI-compatible response grip system (NordicNeuroLab). The position of the risky option (left or right on the screen) was counterbalanced across trials. All human partners were presented in combination with each of the three CE values twice, resulting in 24 human trials and 24 computer trials per run. The feedback video was presented 2 times during a fixed time interval of 2.6 s. The temporal intervals between the decision and outcome stages and the interstimulus intervals between trials varied from 2 to 11 s with a descending probability. All participants completed two runs. Participants received the obtained money from one randomly chosen trial per run. To summarize, this task allowed us to obtain an experimental measure of social avoidance behavior (specifically, the difference in subjective values between engaging in an interaction with a person or a computer) and its associated neural signal (amygdala hyperactivity during social decision-making, amygdala hypersensitivity to human feedback, and reduced reward-associated brain activity in response to positive human feedback). Thus, the task enabled us to concurrently explore behavioral and neural response patterns associated with social avoidance and social feedback processing as core mechanisms underlying the persistence of SA.

Virtual auction task. We further measured the individual monetary value associated with receiving positive or avoiding negative social feedback during a virtual auction task. Specifically, participants were informed that they were participating in a virtual auction against the computer using a random algorithm to invest money. In each trial, a picture of one of six actors indicated which feedback video was being auctioned. The same actors and videos as included in the social gambling task were used plus two additional actors from the same database (see above). In each trial, participants were asked with no imposed time limit to invest any amount of money between 0 € and 1 € at their disposal (in increments of 5 cents) to (1) increase the probability of watching a positive social feedback video or (2) to decrease the probability of watching a negative social feedback video. There were six trials in the positive and six trials in the negative feedback conditions. After completion of all

trials, one trial was chosen randomly and the invested money was compared with a randomly selected amount representing the money invested by the computer. The player (participant or computer) who invested more money won the auction, received the outcome of the trial, and kept the remaining money (1 € minus the invested money). As the investments of the computer were based on uniformly distributed random investments between 0 € and 1 €, each cent invested by the participant corresponded to a probability change of 1% to win the auction. In the positive feedback condition, a positive social feedback video (expressing admiration) was presented if the participant won the auction, while no video was presented if the participant lost. In the negative feedback condition, a negative social feedback video (expressing condescension) was presented if the participant lost and no video was shown if the participant won. If the participants lost, they kept 1 €, regardless of the invested money. The feedback videos were repeated until the participants pressed any key. Notably, winning the auction was associated with a smaller monetary payout than losing the auction. This way, the virtual auction task enabled us to explore whether receiving positive social feedback or avoiding negative feedback would be worth a higher monetary loss for HL compared with LL participants.

fMRI data acquisition and preprocessing. All fMRI data were acquired using a 3T Siemens TRIO MRI system (Siemens) with a Siemens 32-channel head coil. Functional data of the social gambling task were acquired using a T2*-weighted EPI sequence with a TR of 2500 ms, a TE of 30 ms, ascending slicing, a matrix size of 96×96, 37 axial slices with a voxel size of $2 \times 2 \times 3$ mm^3 and a slice thickness of 3.0 mm, a distance factor of 10%, an FOV of 192×192 mm^2, and a flip angle of 90°. High-resolution T1-weighted structural images were collected on the same scanner (TR = 1660 ms, TE = 2.54 ms, matrix size: 256×256, voxel size: $0.8 \times 0.8 \times 0.8$ mm^3, slice thickness = 0.8 mm, FOV = 256×256 mm^2, flip angle = 9°, 208 sagittal slices). To control for inhomogeneity of the magnetic field, fieldmaps were obtained for the T2*-weighted EPI sequence (TR = 392 ms, TE [1] = 4.92, TE [2] = 7.38, matrix size: 64×64, voxel size: $3 \times 3 \times 3$ mm^3, slice thickness = 3.0 mm, distance factor = 10%, FOV = 192×192 mm^2, flip angle = 60°, 37 axial slices). For preprocessing, standard procedures of SPM12 (Wellcome Trust Center for Neuroimaging; https://www.fil.ion.ucl.ac.uk/spm/) implemented in MATLAB (The MathWorks) were used. The first five volumes of each functional time series were removed to allow for T1 signal equilibration before affine registration was used to correct for head movements between scans. Images were initially realigned to the first image of the time series and then re-realigned to the mean of all images. For unwarping, the voxel displacement map (VDM file) was applied to the EPI time series to correct for signal distortion based on B0-field inhomogeneity. Normalization parameters as determined by segmentation and nonlinear warping of the structural scan to reference tissue probability maps in MNI space were applied to all functional images. All images were resampled at $2 \times 2 \times 2$ mm^3 voxel space and spatially smoothed by using a 6 mm FWHM Gaussian kernel. A high-pass filter with a cutoff period of 128 s was used to detrend raw time series.

Behavioral data analysis. Behavioral data were analyzed in SPSS 24 (IBM). Specifically, to analyze the social gambling task, we calculated mixed-design ANOVAs with the estimated CE50 values, the proportion of safe decisions during the behavioral and the fMRI task, and the pleasantness ratings of the feedback videos as dependent variables. For all analyses, group (HL vs LL) served as between-subject factor and the partner condition (human vs computer) was included as within-subject factor. Offered payoffs as safe option were further included as within-subject factor for the behavioral task (0-3 € in steps of 50 cents) and the fMRI task (CE20, CE50, CE80) to analyze the proportion of safe decisions, whereas the analysis of the pleasantness ratings of the feedback videos included the additional within-subject factor feedback valence (positive vs negative feedback). For task validation, we first tested whether we were able to replicate task effects reported by Schultz et al. (2019). Thus, we examined whether increasing safe option payoffs were associated with increased proportions of safe decisions in both behavior and fMRI tasks (main effect of payoff), and whether positive feedback was rated as more pleasant compared with negative feedback (main effect of feedback valence). Moreover, we tested whether we could replicate the previously observed negative association between SA (measured by the Liebowitz Social Anxiety Scale [LSAS]) (Liebowitz, 1987) and social engagement in participants unaffected by loneliness (i.e., the LL group). We then examined the hypothesized effects of loneliness on the subjective value of engaging in social situations and explored loneliness effects on the pleasantness ratings of the feedback videos.

For the analysis of the virtual auction task, effects of the valence (positive vs negative video) and group were included as within- and between-subject factors, respectively, in a mixed-design ANOVA with invested money serving as dependent variable. Greenhouse–Geisser corrections were applied in cases of violated assumptions of sphericity as tested by Mauchly's test. All *post hoc t* tests to disentangle interactions were Bonferroni-corrected (p_{cor}). p values < 0.05 (two-tailed) were considered significant.

fMRI data analysis. To analyze the fMRI data, we used a two-stage approach as implemented in SPM12. On the first level, data were modeled using a fixed-effects model. Onsets and durations of eight conditions (risky decision computer, safe decision computer, risky decision human, safe decision human, positive computer feedback, negative computer feedback, positive human feedback, and negative human feedback) were modeled by a stick function convolved with an HRF. Although individual CE values were used during the fMRI task to equalize the number of trials of each condition between both runs, the decisions of the participants and thereby the resulting number of trials of one condition still differed between runs to varying degrees. We thus decided to concatenate time series of both runs (compare Cho et al., 2021). Baseline regressors were added for each run, and the high-pass filter and temporal nonsphericity estimates were adjusted separately for each run. The six movement parameters were included in the design matrix as regressors of no interest. Within-subject contrasts of interest were calculated on the first level and entered to a random-effects model on the second level. For task validation, one-sample *t* tests were calculated across groups (i.e., decision human > decision computer, risky decision human > risky decision computer, safe decision human > safe decision computer, human feedback > computer feedback, positive feedback > negative feedback). Furthermore, whole-brain task effects (e.g., decision human > decision computer) were analyzed across groups after applying an initial cluster-forming height threshold of $p < 0.001$. Additional whole-brain analyses were calculated to examine neural responses during decision-making (risky decision vs safe decision) and feedback processing (positive vs negative human feedback) in the social gambling task. To further validate whether the previously observed association between SA and increased amygdala activation during social decision-making (risky decision human > safe decision human and risky decision human > risky decision computer) and while receiving human feedback (human feedback > computer feedback) could be replicated in our sample, we extracted parameter estimates of the anatomically defined amygdala for these contrasts and correlated the averaged activity across voxels with SA scores. Likewise, we analyzed the association between SA and increased NAcc response to positive human compared with positive computer feedback. To ensure that a replication of SA-related findings was not driven by loneliness, we included only participants of the LL group in this analysis.

We then assessed group-specific response patterns by calculating two-sample *t* tests. Specifically, to probe the hypothesis of increased amygdala activation during social decision-making in HL participants, we compared brain activity during risky decisions involving a human partner between groups (i.e., $\mathrm{HL}_{\text{risky decision human > safe decision human}} > \mathrm{LL}_{\text{risky decision human > safe decision human}}$, $\mathrm{HL}_{\text{risky decision human > risky decision computer}} > \mathrm{LL}_{\text{risky decision human > risky decision computer}}$). Likewise, the hypothesized increased amygdala responsiveness to human feedback ($\mathrm{HL}_{\text{human feedback > computer feedback}} > \mathrm{LL}_{\text{human feedback > computer feedback}}$) and reduced NAcc reactivity to positive human feedback ($\mathrm{LL}_{\text{positive human feedback > positive computer feedback}} > \mathrm{HL}_{\text{positive human feedback > positive computer feedback}}$) were tested. As the behavioral data indicated an altered responsiveness to negative human feedback (see Behavioral results), we explored group differences in response to negative human feedback ($\mathrm{HL}_{\text{negative human feedback > negative computer feedback}} > \mathrm{LL}_{\text{negative human feedback > negative computer feedback}}$). These contrasts were also calculated in the opposite

direction (e.g., LL $_{\text{risky decision human} > \text{risky decision computer}}$ > HL $_{\text{risky decision human} > \text{risky decision computer}}$). The amygdala and NAcc were anatomically defined according to the Wake Forest University PickAtlas (Maldjian et al., 2003, 2004). p values < 0.05 after familywise error (FWE) correction for multiple testing (p_{FWE}) based on the size of the respective ROI were considered significant. Additional explorative whole-brain analyses were calculated to compare brain activation between groups for the contrasts of interest. Parameter estimates of clusters showing significant group effects were extracted and further analyzed in SPSS 24 to disentangle the group × task condition interaction. Behavioral group effects were correlated with parameter estimates of neural group effects by calculating Pearson's product-moment correlations. Five participants were excluded from fMRI analyses because of excessive head movement (>4 mm/° in any direction; $n = 2$), anatomic abnormalities ($n = 1$), technical issues ($n = 1$), or incomplete data ($n = 1$). Furthermore, 3 participants were excluded from analyses of the decision stage as they always chose the risky option for at least 1 of the partners, while 1 participant was excluded from analyses of the feedback stage because no positive human feedback was shown during both runs.

Multivariate pattern analysis. We conducted a multivariate pattern analysis using the Decoding Toolbox (Hebart et al., 2014) as further task validation and to probe the replicability of the previous finding that decisions of the participants could be decoded from amygdala activation (compare Schultz et al., 2019). Notably, rather than reanalyzing the involvement of the amygdala in social decision-making as examined by the univariate task validation, the multivariate pattern analysis was used to verify the involvement of the amygdala in decision-making processes regardless of the specific partner (human or computer). For the decoding analysis, we used non-normalized and unsmoothed data of each participant and included the same conditions and regressors as outlined above in the single-subject fixed-effects models separately for both runs. The participants' decisions (risky or safe decision) were used as independent variables, and parameter estimates of the corresponding first-level regressors were used as features. Using the default parameters of the Decoding Toolbox, we ran a classification searchlight analysis with a 9 mm searchlight radius and trained a support vector machine classifier (LIBSVM) on the data of one run to decode the decision to play or to choose the safe option. The decoding accuracy was tested on the data of the other run, and the resulting individual accuracy maps minus chance (chance = 50% accuracy) were normalized to MNI space and smoothed using a 6 mm FWHM Gaussian kernel. Maps of accuracy minus chance decoding performance were then entered into a random-effects model on the second level and tested against 0 by calculating a one-sample t test across groups. FWE correction was applied based on the size of the anatomically defined amygdala (compare Schultz et al., 2019). Furthermore, we explored whether the amygdala activation-based decision decoding accuracy during general decision-making (i.e., across human and computer partners) differed between groups by calculating a two-sample t test.

Functional connectivity analyses. Given that social decision-making and the processing of social rewards rely on complex neural networks rather than on single brain regions (Ruff and Fehr, 2014) and given previously reported associations between SA and altered functional connectivity between the involved brain regions (i.e., amygdala or NAcc) and other brain regions (Schultz et al., 2019), we searched for loneliness-related changes in functional connectivity with the same seed regions (amygdala or NAcc) and other brain regions. Contrasts revealing significant group effects in the univariate activity analyses (see above) were thus examined by exploratory generalized psychophysiological interaction (gPPI) analyses using the CONN toolbox 19.b (www.nitrc.org/projects/conn, RRID:SCR_009550). Following the recommendations of the CONN toolbox, preprocessing for the gPPI analyses additionally included a denoising pipeline. Outlier scans were detected by the integrated artifact detection toolbox-based identification using conservative settings (i.e., thresholds of 0.5 mm framewise displacement and 3 SDs above global BOLD signal changes were used) and treated as regressors of no interest in the following analyses. The default denoising pipeline implemented a linear regression of confounding effects of the first five principal noise components from white matter and CSF template masks, 12 motion parameters, scrubbing, and constant task-related effects. A high-pass filter of 0.008 Hz was applied to minimize the effects of physiological and motion-related noise. Regions associated with group effects (amygdala or NAcc) served as seed regions in a seed-to-voxel analysis. The interaction terms of the psychological (task conditions convolved with a canonical HRF) and the physiological factor (blood oxygenation level-dependent signal) were computed for each participant on the first level. The relative measure of connectivity compared with the implicit baseline was calculated by using bivariate regression measures. Connectivity was compared between groups on the second level by using mixed-design ANOVAs.

Bayesian analyses. The main purpose of the current study was to investigate whether HL participants differ from LL participants in variables associated with core etiologic mechanisms of SA. While frequentist analyses allow to interpret the significance of an observed group difference, a nonsignificant result cannot be interpreted as evidence for the equivalence of groups (Keysers et al., 2020). However, evidence for comparable neural responses to social stimuli and during social decision-making in HL and LL participants would have important clinical implications as this would indicate that loneliness is not associated with neurobiological mechanisms of SA, which are the targets of cognitive-behavioral therapy manuals. Importantly, Bayesian analyses are able to distinguish between the absence of evidence (i.e., more data are needed to interpret the results) and evidence for the absence of an effect and are thus recommended to complement frequentist analyses (Keysers et al., 2020). Therefore, for all hypothesized differences between HL and LL participants that could not be confirmed by classical inference analyses, Bayesian t tests were conducted to quantify the evidence for the null hypotheses (i.e., HL participants do not differ from LL participants) using the default settings for two-tailed independent t tests implemented in JASP (JASP Team, 2020). Specifically, group differences in the subjective value of engaging in social situations during the social gambling task (i.e., the individual CE50 for human partners minus CE50 for the computer partner) and pleasantness ratings of positive human feedback (minus the ratings of positive computer feedback) were reanalyzed by calculating Bayesian t tests. Moreover, as we expected HL participants to differ from LL participants regarding amygdala responsiveness to risky decisions involving a human partner, parameter estimates of the anatomically defined amygdala response during the decision stage were averaged across all voxels and reanalyzed to quantify evidence of differences between groups for the following contrasts of interest: risky decision human > risky decision computer and risky decision human > safe decision human. Likewise, parameter estimates of activation during the feedback stage were extracted from the amygdala to reanalyze responsiveness to human feedback (compared with computer feedback). To reanalyze reward-associated brain activity in response to positive human feedback (compared with computer feedback), parameter estimates were extracted from the NAcc.

Mediation and moderation analyses. For variables that were found to be associated with SA in the LL group, we calculated moderation analyses to investigate whether group (HL vs LL) moderated the size of SA effects. A significant interaction between group and SA would thus indicate that the association between SA and the dependent variable would differ between HL and LL participants. Moderation analyses were calculated for amygdala activation during social decision-making (risky decision human > safe decision human and risky decision human > risky decision computer) and for the subjective values of engaging in social situations as dependent variables, SA scores as independent variable, and group as moderator. Again, parameter estimates were averaged across all voxels of the anatomic amygdala.

Likewise, we conducted moderation analyses to examine whether the differences in negative feedback processing between HL and LL participants differed as a function of SA (i.e., whether the associations between loneliness and the dependent variables were weakened or enhanced for participants with high SA scores). Thus, group (HL or LL) was used as independent variable to analyze those dependent variables that showed differences between groups, and SA was included as moderator variable. In addition to the investigation of interaction effects between group and SA, we examined whether the observed differences between HL and LL

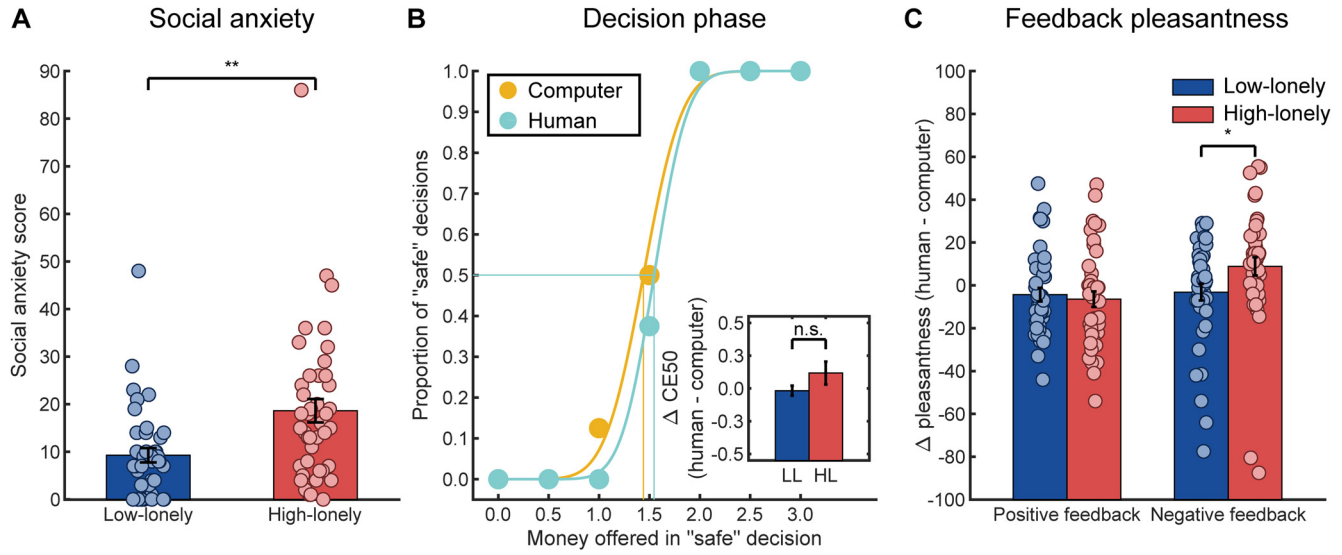

Figure 2. Behavioral results of the decision and feedback phase of the social gambling task. ***A***, Participants with HL showed significantly increased SA scores as assessed with the LSAS. ***B***, The proportion of safe decisions during the social gambling task increased with higher payoffs offered in those safe decisions (main effect of offered payoff for the behavioral task: $F_{(2.95,236.14)} = 183.77$, $p < 0.001$, $\eta_p^2 = 0.70$; fMRI task: $F_{(2,158)} = 185.43$, $p < 0.001$, $\eta_p^2 = 0.70$; example data of the behavioral task from 1 HL participant are presented). As presented in the inlay, HL participants did not significantly differ from participants with LL with regard to the subjective value of engaging in a social situation (i.e., CE50, the payoff offered in the safe option associated with 50% of safe decisions; $t_{(47.81)} = 1.42$, $p = 0.16$, Bayes factor [BF_{10}] = 0.57). ***C***, By contrast, groups significantly differed in their pleasantness ratings of the negative feedback videos. Compared with the negative computer feedback video, HL participants rated the negative human feedback video as more pleasant, whereas LL participants showed the opposite pattern of ratings. No differences between groups were observed for positive feedback. Each marker in ***B*** represents the mean of 8 trials. Bars represent group means. Error bars indicate SEM. *$p < 0.05$. **$p < 0.01$. n.s., not significant.

participants could be explained by increased SA in HL participants. Therefore, mediation analyses were calculated with group serving as independent variable and SA serving as mediator.

To examine the influence of further possible confounding variables on significant group effects (i.e., depressive symptomatology assessed by the Beck's Depression Inventory II [BDI], Beck et al., 1996; and childhood maltreatment assessed by the Childhood Trauma Questionnaire [CTQ], Bernstein et al., 1994), we calculated mediation and moderation analyses using the PROCESS macro version 3.4 for SPSS (Hayes, 2017). BDI and CTQ scores were used as mediator and moderator variables and group as predictor variable. Again, mediation analyses were calculated to examine whether observed differences between groups might be driven by differences in psychiatric symptomatology, whereas moderation analyses were conducted to investigate a potential interaction of loneliness (HL vs LL) with the moderation variable. For mediation analyses, 10,000 bootstrap samples were used. Variables were mean-centered before calculating moderation analyses. Mediations were considered significant if the 95% CI of an indirect effect excluded zero, while moderations were considered significant if $p < 0.05$ for the interaction effect of group with the potential moderator. Moreover, we further examined whether the observed effects of group remained significant ($p < 0.05$ for the direct effect of group) after including the potential confounding variables (SA, BDI, and CTQ scores) as covariates in the regression model to probe the robustness of the observed explorative loneliness-related findings.

Results

Behavioral results

As expected, SA was significantly increased in HL participants ($t_{(67.74)} = 3.25$, $p = 0.002$, $d = 0.72$; mean LSAS score ± SD in HL: 18.64 ± 15.91, range: 0-86; LL: 9.28 ± 9.56, range: 0-48; Fig. 2*A*) (Lieberz et al., 2021), and task effects of the social gambling task reported by Schultz et al. (2019) were replicated across groups: the proportion of safe decisions in the behavioral social gambling task significantly increased with higher payoffs offered as safe alternative to the risky gambling decision across groups (main effect of offered payoff: $F_{(2.95,236.14)} = 183.77$, $p < 0.001$, $\eta_p^2 = 0.70$; Fig. 2*B*) and was highest for an offered payoff of 3 € (mean proportion of safe decisions ± SD for an offered payoff of 0 €: 8.16 ± 17.06%; 0.5 €: 8.38 ± 16.44%; 1 €: 19.36 ± 28.44%; 1.5 €: 37.96 ± 36.12%; 2 €: 76.98 ± 30.70%; 2.5 €: 84.98 ± 25.85%; 3 €: 88.11 ± 23.48%). Likewise, the proportion of safe decisions differed between all three payoffs offered during the fMRI implementation of the task (main effect of offered payoff: $F_{(2,158)} = 185.43$, $p < 0.001$, $\eta_p^2 = 0.70$; *post hoc* comparisons: CE20 vs CE50: $t_{(80)} = 8.27$, $p_{cor} < 0.001$, $d = 1.08$; CE50 vs CE80: $t_{(80)} = 11.02$, $p_{cor} < 0.001$, $d = 1.44$; mean proportion of safe decisions ± SD for an offered payoff of CE20: 12.13 ± 18.91%; CE50: 41.57 ± 32.27%; CE80: 82.30 ± 22.69%). Importantly, as individual payoffs were calculated for the fMRI task separately for human and computer partners to equalize the ratio of risky and safe decisions, the likelihood of safe decisions during fMRI differed neither between partners nor between groups (HL vs LL) (all main effects or interactions of the partner condition or group F values < 1.48, p values > 0.05). As intended, positive feedback videos were rated as more pleasant than negative ones (main effect of feedback valence: $F_{(1,80)} = 174.73$, $p < 0.001$, $\eta_p^2 = 0.69$). SA was indeed negatively associated with the subjective value of engaging in social situations in the LL group, but the correlation failed to reach significance ($r_{(38)} = -0.22$, $p = 0.21$).

However, contrary to previously observed effects of SA (Schultz et al., 2019), loneliness (HL vs LL) affected neither the subjective value of engaging in social situations during the behavioral social gambling task nor investments in the virtual auction task (all p values > 0.05). Nevertheless, analyses of pleasantness ratings of the feedback videos revealed a significant interaction of group × partner × feedback valence ($F_{(1,80)} = 4.02$, $p = 0.048$, $\eta_p^2 = 0.05$). To disentangle the interaction, we calculated further mixed-design ANOVAs separately for the positive and negative feedback videos. Surprisingly, no group effects were observed for positive feedback (all p values > 0.05), but we found a significant interaction of group

× partner for negative feedback ($F_{(1,80)} = 4.34$, $p = 0.04$, $\eta_p^2 = 0.05$; Fig. 2*C*): HL participants rated the negative human feedback as more pleasant compared with the negative computer feedback ($t_{(41)} = 2.09$, $p_{cor} = 0.09$), while LL participants showed the opposite pattern of ratings ($t_{(39)} = -0.82$, $p_{cor} = 0.84$). Two additional explorative *post hoc* tests indicated that HL participants rated the negative computer feedback as less pleasant compared with LL participants (HL vs LL: $t_{(80)} = -2.09$, $p_{cor} = 0.08$; mean pleasantness ratings ± SD in HL participants: 25.91 ± 22.94; LL: 36.85 ± 24.38), whereas group differences vanished when negative feedback was provided by a human partner ($t_{(80)} = 0.34$, $p_{cor} \approx 1.00$; mean pleasantness ratings ± SD in HL participants: 34.77 ± 15.28; LL: 33.68 ± 14.29).

fMRI results

Multivariate and univariate analyses of neural activation across groups replicated all previous task effects (Schultz et al., 2019). As such, a linear support vector machine classifier based on amygdala activation was able to decode the decision (risky vs safe) significantly better than chance (mean accuracy ± SD = 53.64 ± 9.07%; 30, −4, 28, $t_{(73)} = 3.45$, $p_{FWE} = 0.048$). Amygdala activation increased during decisions involving a human partner compared with the computer partner (right: 22, −6, −12, $t_{(73)} = 3.68$, $p_{FWE} = 0.03$; left: −22, −8, −12, $t_{(73)} = 4.00$, $p_{FWE} = 0.01$). Specifically, amygdala activity was enhanced during trials in which participants chose the risky option with a human partner compared with the computer partner (right: 22, −6, −12, $t_{(73)} = 4.58$, $p_{FWE} = 0.002$; left: −22, −8, −12, $t_{(73)} = 4.23$, $p_{FWE} = 0.006$; Fig. 3*A*), while no differences in amygdala activity between partners were observed for safe decisions. Moreover, receiving feedback from the human partner activated the amygdala significantly stronger than computer feedback (right: 22, −6, −14, $t_{(75)} = 9.67$, $p_{FWE} < 0.001$, left: −22, −8, −12, $t_{(75)} = 9.66$, $p_{FWE} < 0.001$), and NAcc activity was increased in response to positive feedback compared with negative feedback across partner types (right: 12, 8, −6, $t_{(75)} = 6.45$, $p_{FWE} < 0.001$, left: −14, 10 −10, $t_{(75)} = 4.91$, $p_{FWE} < 0.001$). Notably, while we found no association between SA and feedback processing, we were able to replicate the previously observed association between SA and amygdala hyperactivity during social decision-making in the LL group (SA scores correlated with right amygdala activity for risky decision human > risky decision computer: $r_{(35)} = 0.41$, $p = 0.01$; risky decision human > safe decision human: $r_{(35)} = 0.44$, $p = 0.007$). For whole-brain task effects, see Tables 1 and 2.

Importantly, however, neither amygdala activation during the decision or feedback stage nor the accuracy of decoding risky versus safe decisions based on amygdala activation patterns significantly differed between HL and LL participants. Conversely, we observed significant differences in striatal responses to the feedback videos: HL participants showed significantly smaller NAcc responses to human (vs computer) feedback than LL individuals (14, 14, −10, $t_{(74)} = 3.07$, $p_{FWE} = 0.02$). Again, the group difference was specific for negative feedback (14, 14, −10, $t_{(74)} = 3.21$, $p_{FWE} = 0.01$; Fig. 3*B*), whereas no significant group effects were observed for responses to positive feedback. *Post hoc* tests revealed increased NAcc responsiveness to negative human feedback compared with the computer feedback in LL participants ($t_{(36)} = 2.59$, $p_{cor} = 0.03$, $d = 0.53$), while HL participants exhibited the opposite response pattern ($t_{(38)} = -1.96$, $p_{cor} = 0.12$). In line with the behavioral results, further explorative *post hoc* tests indicated that group differences were based on a significantly enhanced NAcc responsiveness to the negative computer feedback in HL participants (HL vs LL: $t_{(74)} = 2.80$, $p_{cor} = 0.01$, $d = 0.62$), whereas group differences showed the opposite tendency for responses to negative human feedback ($t_{(74)} = -0.98$, $p_{cor} = 0.64$). No further group differences in brain activity were observed.

Exploratory gPPI analyses of the negative feedback condition with the NAcc serving as seed region indicated enhanced functional connectivity of the left NAcc with a cluster including the hippocampus in HL compared with LL participants (−14, −22, −14, $k = 73$, $t_{(74)} = 5.38$, $p_{FWE} = 0.049$ on cluster level; Fig. 4). Again, *post hoc* tests revealed an opposing pattern between groups when receiving negative human (vs computer) feedback: enhanced connectivity in HL participants ($t_{(38)} = 3.06$, $p_{cor} = 0.01$, $d = 0.63$) but reduced connectivity in LL participants ($t_{(36)} = -4.93$, $p_{cor} < 0.001$, $d = -1.15$). Two further *post hoc* comparisons again revealed differences between groups for negative computer feedback as functional connectivity was significantly reduced in HL participants (HL vs LL: $t_{(74)} = -4.62$, $p_{cor} < 0.001$, $d = 1.06$), whereas the involvement of a human partner reversed this pattern with significantly increased functional connectivity in HL participants (HL vs LL: $t_{(74)} = 2.40$, $p_{cor} = 0.04$, $d = 0.55$). Interestingly, NAcc-hippocampus connectivity not only correlated with NAcc responses to negative human feedback (contrasted with negative computer feedback: $r_{(74)} = -0.33$, $p = 0.004$, i.e., increased connectivity was associated with reduced neural reactivity), but also with pleasantness ratings of negative feedback videos ($r_{(74)} = 0.23$, $p = 0.04$; Fig. 4). The correlation between NAcc activity and negative feedback ratings was similar but failed to reach significance ($r_{(74)} = -0.20$, $p = 0.09$).

Bayesian analyses

Bayesian analyses revealed moderate evidence for the absence of group differences in variables that have previously been associated with SA (compare Schultz et al., 2019), with our data being at least 3 times more likely under the null hypothesis (H0: no differences between groups) than under the alternative hypothesis (HL differ from LL participants in any direction). Specifically, Bayesian *t* tests revealed moderate evidence that HL participants indeed did not differ from LL participants regarding the pleasantness ratings of positive human feedback as our data were found to be almost 4 times more likely under the H0 than under the alternative hypothesis (Bayes factor (BF_{10}) = 0.25, median effect size = 0.08, 95% credible interval: [−0.32, 0.49]).

Likewise, Bayesian analyses revealed moderate evidence that groups showed equal reward-associated brain activity in response to positive human feedback (left NAcc: $BF_{10} = 0.25$, median effect size = 0.07, 95% credible interval: [−0.35, 0.49]; for the right NAcc, the evidence is inconclusive: $BF_{10} = 0.43$, median effect size = 0.23, 95% credible interval: [−0.19, 0.66]) and moderate evidence in favor of the H0 for amygdala reactivity to human feedback (left: $BF_{10} = 0.24$, median effect size = −0.004, 95% credible interval: [−0.42, 0.41]; right: $BF_{10} = 0.24$, median effect size ≈ 0.00, 95% credible interval: [−0.42, 0.42]). The same pattern of results was observed for amygdala activation during the decision stage of the social gambling task as our data were up to 4 times more likely under the assumption of comparable activation between groups (H0) than under the alternative hypothesis (left amygdala activation for risky decisions with a human partner compared with a computer partner: $BF_{10} = 0.24$, median effect size = 0.03, 95% credible interval: [−0.39, 0.45]; left amygdala activation for risky decisions with a human partner contrasted with safe decisions in trials with a human partner: $BF_{10} = 0.33$, median effect size = −0.17, 95% credible interval: [−0.61, 0.25]; right: $BF_{10} = 0.24$, median effect size = −0.01, 95% credible interval: [−0.43, 0.41]). For right amygdala activation, there was insufficient evidence to draw a conclusion for or against the

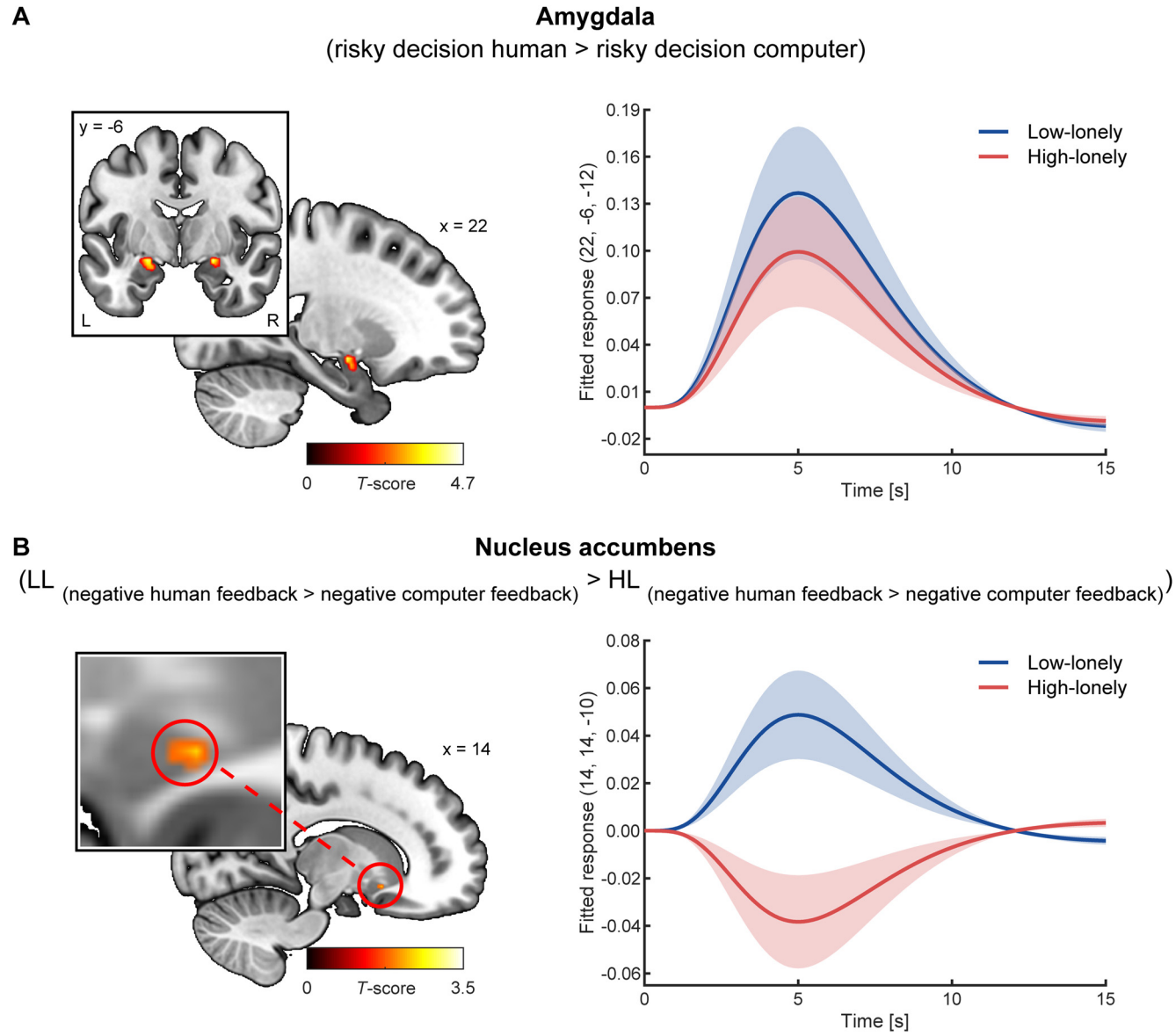

Figure 3. Neural activation during the social gambling task. ***A***, Amygdala activity was significantly enhanced during the decision phase of the social gambling task when participants chose the risky option with a human partner compared with the computer partner (right: 22, −6, −12, $t_{(73)} = 4.58$, $p_{FWE} = 0.002$; left: −22, −8, −12, $t_{(73)} = 4.23$, $p_{FWE} = 0.006$). In line with the behavioral results, no group differences in neural activity were observed during the decision phase. ***B***, During the feedback stage, participants with HL showed attenuated NAcc responses to negative feedback given by human partners compared with the computer partner. In contrast, NAcc reactivity to negative human feedback was enhanced compared with computer feedback in participants with LL. Shaded areas represent the SEM of the fitted responses based on the HRF. For illustration purpose, clusters are shown with significance levels of $p < 0.05$ uncorrected. L, left; R, right.

hypothesis that groups exhibit equal responsiveness to risky decisions involving a human partner (contrasted with the computer; $BF_{10} = 0.50$, median effect size = 0.26, 95% credible interval: [−0.16, 0.70]). However, descriptive analyses revealed an opposing response pattern in HL participants to what has been expected because of increased SA symptoms: while LL participants showed slightly enhanced amygdala activation (mean parameter estimates ± SD: 0.25 ± 1.06), amygdala activation was reduced in HL participants (mean parameter estimates ± SD: −0.02 ± 0.68; compare Fig. 3*A*). Likewise, no evidence for any of the hypotheses (null or alternative hypothesis) was observed for the subjective value of engaging in social situations ($BF_{10} = 0.57$, median effect size = −0.29, 95% credible interval = [−0.74, 0.15]). Again, descriptive analyses revealed enhanced values of social engagement in HL compared with LL participants, which is contrary to the previously reported negative association with SA (Fig. 2*B*, inlay) (compare Schultz et al., 2019).

Regarding the invested money during the virtual auction task, Bayesian analyses provided moderate evidence for comparable investments between groups to avoid negative human feedback ($BF_{10} = 0.33$, median effect size = 0.17, 95% credible interval = [−0.23, 0.59]) or to receive positive human feedback ($BF_{10} = 0.33$, median effect size = 0.18, 95% credible interval = [−0.23, 0.59]).

Interactions of loneliness with SA

To summarize, although HL individuals reported higher SA scores, loneliness was not associated with behavioral and neural correlates, which have been previously found to be affected by SA and which could be partially replicated in LL participants. We thus explored whether SA-related findings differed significantly between HL and

Table 1. Whole-brain findings during decision-making across groups[a]

Region	Right/left	Cluster size (voxel)	Peak *T*	MNI coordinates		
				x	*y*	*z*
Decision human > decision computer						
Medial orbitofrontal gyri	Bilateral	351	6.28	2	44	−14
Precuneus	Bilateral	800	6.04	4	−56	28
Risky decision > safe decision						
Inferior frontal gyrus, triangularis	R	2218	8.77	44	24	24
Middle occipital gyrus	L	588	7.65	−44	−68	4
Fusiform gyrus	L	249	7.29	−22	−80	−8
Middle temporal gyrus	R	452	6.77	42	−58	10
Lingual gyrus	R	595	6.60	4	−80	−4
Anterior cingulate cortex	Bilateral	331	6.26	8	−14	30
Precentral gyrus	L	557	6.15	−42	−6	48
Supplementary motor area	R	633	6.09	8	8	60
Supramarginal gyrus	R	313	6.07	44	−40	14
Superior parietal gyrus	L	203	5.99	−26	−52	48
Superior temporal gyrus	R	110	5.90	50	−22	−4
Inferior temporal gyrus	L	120	5.73	−40	−44	−14
Superior occipital gyrus	L	220	5.58	−14	−66	38
Insular cortex	L	214	5.47	−30	26	2
Inferior parietal gyrus	R	139	5.28	28	−52	52
Risky decision human > risky decision computer						
Superior temporal gyrus	R	448	7.60	48	−40	10
Precuneus	Bilateral	496	6.64	6	−56	28
Medial orbitofrontal gyri	Bilateral	328	5.79	2	42	−14
Inferior frontal gyrus, triangularis	R	315	5.49	42	16	22

[a]Cluster sizes are based on the initial cluster-forming height threshold of $p < 0.001$. Peak *T* and MNI coordinates are listed for FWE-corrected *p* values < 0.05 on peak level. No cluster survived the FWE correction on the peak level for the safe decision human > safe decision computer contrast.

Table 2. Whole-brain findings during the feedback phase across groups[a]

Region	Right/left	Cluster size (voxel)	Peak *T*	MNI coordinates		
				x	*y*	*z*
Human feedback > computer feedback						
Middle temporal gyrus	R	6837	12.07	54	−40	8
Calcarine fissure	R	141	12.01	22	−94	−2
Amygdala	L	3273	9.66	−22	−8	−12
Fusiform gyrus	R	361	9.29	40	−48	−16
Fusiform gyrus	L	296	8.44	−38	−48	−20
Middle occipital gyrus	L	32	7.65	−20	−94	−2
Gyri rectus	Bilateral	295	6.54	6	38	−16
Inferior occipital gyrus	R	42	5.29	44	−76	−6
Positive feedback > negative feedback						
Inferior occipital gyrus	R	341	8.32	26	−92	−2
Caudate nuclei	Bilateral	2792	8.10	8	10	−2
Middle cingulate gyri	Bilateral	2897	6.80	−2	−34	34
Inferior occipital gyrus	L	101	6.63	−28	−88	−6
Angular gyrus	L	3721	6.15	−40	−66	46
Middle frontal gyrus	L	2771	6.11	−30	16	52
Precentral gyrus	R	2059	5.62	36	−28	62
Superior frontal gyrus	R	722	5.59	20	34	48
Inferior orbitofrontal gyrus	L	55	5.53	−26	30	−16
Fusiform gyrus	L	229	5.43	−26	−46	−18
Positive human feedback > negative human feedback						
Caudate nuclei	Bilateral	685	7.52	8	10	−2
Angular gyrus	L	937	6.23	−40	−68	34
Middle temporal gyrus	R	1487	6.09	56	−36	6
Middle temporal gyrus	L	551	5.63	−58	−42	10
Middle temporal gyrus	L	280	5.47	−48	−70	6
Precentral gyrus	R	1087	5.31	40	−26	64

[a]Cluster sizes are based on the initial cluster-forming height threshold of $p < 0.001$. Peak *T* and MNI coordinates are listed for FWE-corrected *p* values < 0.05 on peak level. For the positive feedback > negative feedback contrast, the NAcc is included in the caudate nuclei cluster.

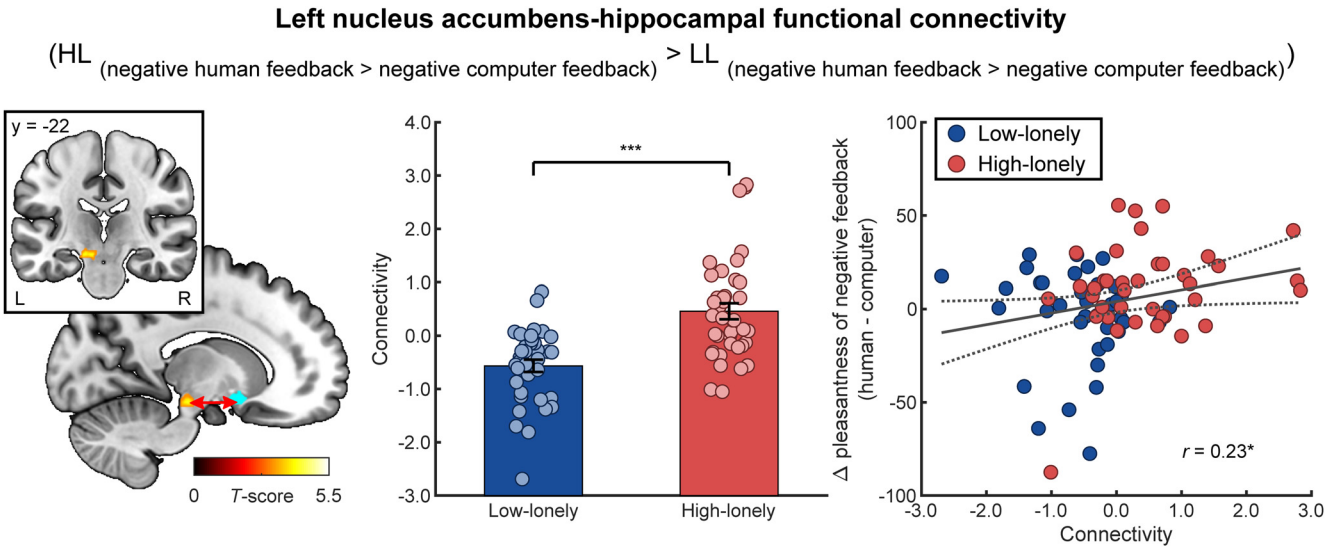

Figure 4. Functional connectivity during the social gambling task. Participants with HL showed enhanced functional connectivity of the NAcc (blue sphere) with a cluster including the hippocampus while receiving negative human (vs computer) feedback compared with participants with LL. Functional connectivity positively correlated with the pleasantness ratings of the negative human feedback (compared with the negative computer feedback). Dashed line indicates the 95% CI of the plotted regression line. Bars represent group means. Error bars indicate SEM. $^{*}p < 0.05$. $^{***}p < 0.001$. L, left; R, right.

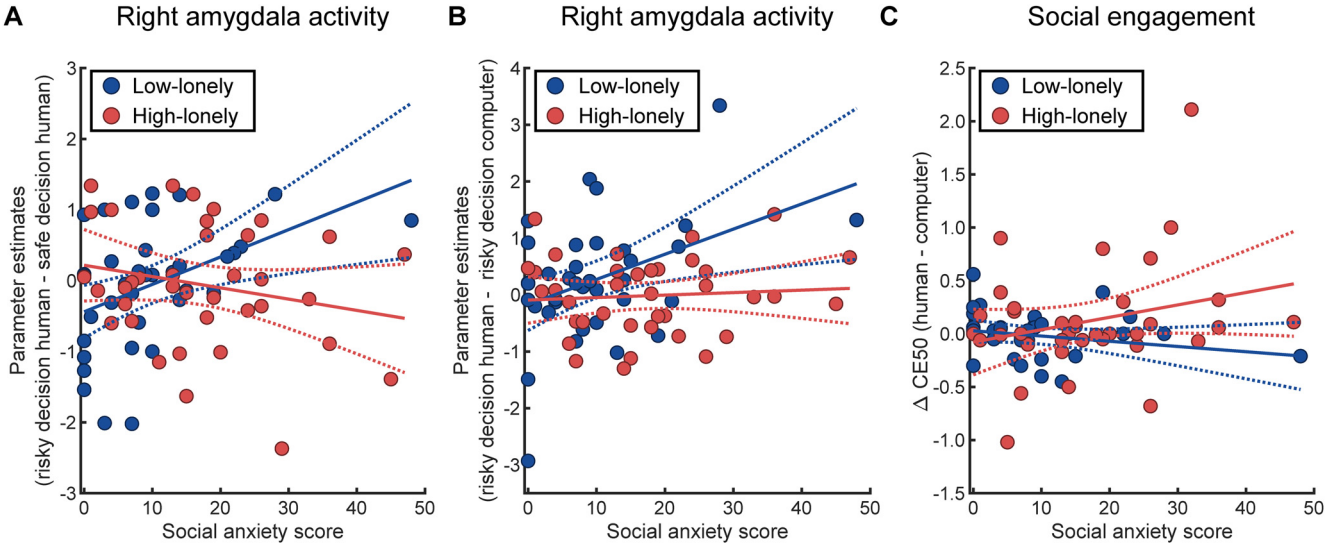

Figure 5. Interactions of loneliness with SA. ***A***, Moderation analyses revealed that the positive association of SA with right amygdala activation during risky social decision-making (risky decision human – safe decision human) as observed in participants with LL ($\beta = 0.63$, $p = 0.007$, 95% CI: [0.18, 1.08]) was not evident in participants with HL ($\beta = -0.26$, $p = 0.17$, 95% CI: [−0.63, 0.11]). ***B***, Likewise, the positive relationship of SA with right amygdala activation during social decision-making contrasted with risky decisions involving a computer partner vanished in the HL group (LL: $\beta = 0.69$, $p = 0.003$, 95% CI: [0.25, 1.14]; HL: $\beta = 0.07$, $p = 0.72$, 95% CI: [−0.30, 0.43]). ***C***, Moreover, the nonsignificant negative association of SA with the subjective value of engaging in a social situation (i.e., CE50, the payoff offered in the safe option associated with 50% of safe decisions) in the LL group ($\beta = -0.17$, $p = 0.48$, 95% CI: [−0.64, 0.30]) was reversed in the HL group ($\beta = 0.40$, $p = 0.049$, 95% CI: [0.002, 0.80]). Thus, higher SA symptomatology was even associated with increased subjective values of engaging in social situations for participants suffering from loneliness. Dashed lines indicate the 95% CI of the plotted regression lines.

LL participants. Indeed, moderation analyses revealed that SA-related effects on amygdala activation during social decision-making were significantly different for HL compared with LL participants (interaction of SA with group for right amygdala activation during risky decisions with a human partner compared with safe decisions with a human partner: $\beta = -0.88$, $t_{(70)} = -3.02$, $p = 0.004$, 95% CI: [−1.47, −0.30]; for right amygdala activation during risky decisions with a human partner compared with risky decisions with the computer partner: $\beta = -0.63$, $t_{(70)} = -2.16$, $p = 0.03$, 95% CI: [−1.20, −0.05]; Fig. 5*A*,*B*). As already reported (see fMRI results), SA was positively associated with the average activation across all voxels of the right amygdala for risky decisions involving a human partner (compared with safe decisions involving a human partner: $\beta = 0.63$, $p = 0.007$, 95% CI: [0.18, 1.08]; compared with risky decisions involving the computer: $\beta = 0.69$, $p = 0.003$, 95% CI: [0.25, 1.14]) in LL participants. Conversely, this association vanished in the HL group (risky decisions involving a human partner vs safe decisions involving a human partner: $\beta = -0.26$, $p = 0.17$, 95% CI: [−0.63, 0.11]; risky decisions involving a human partner vs risky decisions involving a computer partner: $\beta = 0.07$, $p = 0.72$, 95% CI: [−0.30, 0.43]). Moreover, moderation analyses indicated that the association of SA with the subjective values of engaging in social situations

might be altered in HL participants (interaction of SA with group: $\beta = 0.57$, $t_{(67)} = 1.84$ $p = 0.07$, 95% CI: [−0.05, 1.18]; Fig. 5*C*). As such, the reported nonsignificant negative association of SA with the social engagement in the LL group ($\beta = -0.17$, $p = 0.48$, 95% CI: [−0.64, 0.30]; see also Behavioral results) was reversed in the HL group ($\beta = 0.40$, $p = 0.049$, 95% CI: [0.002, 0.80]). Thus, higher SA symptomatology was significantly associated with increased subjective values of engaging in social situations for participants suffering from loneliness.

We then probed whether the differences between HL and LL participants were based on increased SA score in HL participants or whether loneliness effects on the processing of negative feedback differed for participants with high or low SA scores. Importantly, the observed effects of loneliness (HL vs LL) on NAcc responsiveness to negative human feedback (vs negative computer feedback) and on the NAcc-hippocampal functional connectivity while receiving negative feedback remained significant after including SA scores as covariate in the regression models (p values < 0.01 for all direct effects of group after including SA). Furthermore, no significant interactions between group and SA were observed, indicating that an altered processing of negative feedback in HL participants was not enhanced or diminished by increased SA symptomatology. Finally, we explored whether the altered feedback processing in HL participants was driven by increased SA by calculating mediation analyses with SA scores as potential mediator. Results revealed that none of the reported group effects was driven by SA. Conversely, analyses showed a significant suppressor effect of SA on the relationship between group and NAcc responses (indirect effect of group on NAcc activity via SA: $\beta = 0.14$, SE = 0.10, 95% CI: [0.005, 0.40]). Thus, the absolute height of the group effect even increased after including SA as mediator (effect of group without taking SA into account: $\beta = -0.69$, SE = 0.22, 95% CI: [−1.12, −0.26]; with SA as mediator: $\beta = -0.83$, SE = 0.23, 95% CI: [−1.28, −0.38]; for NAcc-hippocampal functional connectivity and pleasantness ratings of negative human vs computer feedback, 95% CIs included zero for the SA mediator effect; i.e., the indirect effect of group via SA).

Effects of further confounding variables

Groups differed significantly regarding psychiatric symptoms (compare Lieberz et al., 2021). In addition to increased SA symptomatology, HL participants reported more depressive symptoms ($t_{(50.89)} = 4.15$, $p < 0.001$, $d = 0.92$; mean BDI score ± SD in HL: 6.62 ± 6.76; LL: 2.03 ± 2.31) and more severe childhood maltreatment ($t_{(80)} = 2.38$, $p = 0.02$, $d = 0.53$; mean CTQ score ± SD in HL: 38.86 ± 10.28; LL: 31.90 ± 15.76). Importantly, as reported for SA, the observed effects of loneliness (HL vs LL) on NAcc responsiveness to negative human feedback remained significant after including the depression or childhood maltreatment as covariates in the regression models (p values < 0.01 for all direct effects of group after including the potential confounding variables). Likewise, loneliness effects on NAcc-hippocampal functional connectivity while receiving negative human feedback were found to be robust (all direct effects of group after including the potential confounding variables p values < 0.0001). Mediation and moderation analyses indicated that none of the reported group effects was mediated or moderated by confounding psychiatric symptoms (the 95% CI of all tested indirect effects included zero and all interaction effects of group with the potential moderator p values > 0.05).

Discussion

The current study sought to investigate shared and distinct behavioral and neural response patterns underlying SA and liness. While we were able to replicate previously reported task effects and SA-related amygdala hyperactivation during social decision-making (compare Schultz et al., 2019), our results revealed that a previously observed neurocircuitry underlying avoidance behavior in SA is not evident in lonely individuals. HL participants differed from LL participants neither in the subjective value of engaging in social situations nor in neural responses to social decision-making and positive social feedback. Moreover, the association of SA symptomatology with increased amygdala activation during social decision-making vanished in HL participants. Conversely, the previously reported association of higher SA with reduced subjective values of engaging in social situations was even reversed in HL participants. Further explorative analyses indicated that HL participants showed an altered responsiveness to negative computer feedback as evident in reduced pleasantness ratings and increased striatal activity, which was normalized when negative feedback was provided by a human partner. Moreover, striatal-hippocampal functional connectivity in HL participants, which was diminished while receiving negative computer feedback, was significantly increased during negative social feedback.

Our results indicate that neural and behavioral correlates of loneliness differ from a socially avoidant phenotype associated with SA. Loneliness did not significantly correlate with behavioral tendencies to withdraw from social interactions in the current study. Human and animal research has consistently shown that the amygdala is crucially involved in the processing of threat-related stimuli, and hyperactivation of the amygdala is known as a core mechanism underlying anxiety disorders (Phelps and LeDoux, 2005; Etkin and Wager, 2007). Moreover, amygdala habituation to threat-related stimuli and amygdala connectivity with prefrontal regions predict subsequent avoidance behavior (Björkstrand et al., 2020; Lisk et al., 2020; Mao et al., 2020). Likewise, we have previously found that amygdala activation during decisions in the social gambling task increases with SA symptomatology and negatively correlates with the subjective value to engage in social situations (Schultz et al., 2019). By contrast, the subjective value of engaging in a social situation did not differ between HL and LL participants, and Bayesian analyses revealed evidence for comparable amygdala activation during the decision and feedback stages. Moreover, the link between amygdala activation during social decision-making and SA symptoms differed significantly between HL and LL participants, thus providing further support for the heterogeneity in clinical phenotypes and underlying biotypes of SA (Spokas and Cardaciotto, 2014; Williams, 2017). In line with our findings, neuroanatomical correlates of social avoidance behavior were previously found to be unaffected by loneliness (Tian et al., 2016). This notion is consistent with etiologic theories that highlight maladaptive social cognitions in the development and maintenance of loneliness (Spithoven et al., 2017; Cacioppo and Cacioppo, 2018). Likewise, cognitive-behavioral interventions were found to be more effective in targeting social biases than social skill trainings (Masi et al., 2011; Veronese et al., 2021). There is preliminary evidence that established cognitive-behavioral treatments targeting SA concurrently decrease feelings of loneliness and vice versa (Alfano et al., 2009; Suveg et al., 2017; Haslam et al., 2019; Käll et al., 2021; O'Day et al., 2021), but our findings of distinct behavioral and neural substrates suggest that loneliness-adjusted protocols might improve therapeutic outcomes.

Moreover, our explorative results provide new insights into the neural pathways underlying loneliness. Unexpectedly, striatal

activity during negative social feedback was reduced while pleasantness ratings were increased in HL participants. Notably, activation of the NAcc is associated with goal-directed approach and avoidance behavior and involved in avoiding social punishment (Kohls et al., 2013; Damiano et al., 2015; Floresco, 2015). Furthermore, our results are in line with parcellation studies highlighting specific roles of the ventral-caudal NAcc shell and the rostral, core-like NAcc. The former has been associated with reward anticipation and reward processing, while activation of the latter may also reflect the processing of negative events (Baliki et al., 2013; Xia et al., 2017; Oldham et al., 2018). Concordantly, the observed group differences in response to negative feedback were restricted to rostral, core-like parts of the NAcc, whereas positive feedback activated both rostral and caudal parts of the NAcc across groups. As HL participants rated the negative social feedback videos as more pleasant than the negative computer feedback, reduced core-like NAcc responses to negative social feedback might thus reflect reduced tendencies to avoid this negative social feedback. Conversely, the opposite pattern of results was observed for LL participants. Furthermore, the enhanced functional coupling of the NAcc with a hippocampal cluster that correlated with individual pleasantness ratings is in line with the involvement of this neural circuit in hedonic processing (Yang et al., 2020) and might reflect the rewarding experience of a social feedback for socially deprived individuals (Tomova et al., 2020). As such, our results indicate that HL participants might be more affected by negative events compared with LL participants. The involvement of another human, however, might attenuate this bias. Nevertheless, we have recently found a compromised neural integration of social information in HL participants evident in various brain regions, including the NAcc (Lieberz et al., 2021). Furthermore, loneliness has been associated with a reduced recognition of negative vocal expressions (Morningstar et al., 2020). Thus, the reduced NAcc activity might also reflect diminished differentiation between positive and negative feedback, resulting in a dysregulated reward system responsiveness to negative social stimuli as observed for the NAcc-hippocampus connectivity. However, inference about cognitive processes from neural activation should always be drawn with restraint (Poldrack, 2006), and results regarding biased emotion recognition in loneliness are inconclusive (Spithoven et al., 2017). Future studies are warranted to further investigate the impact of loneliness on the processing of negative events in general and on the processing of negative social feedback in particular. For instance, implementing representational similarity analyses and incorporating multimodal data might help to understand how negative social feedback is represented in HL participants, how its processing contributes to future behavior, and whether its neural representation differs from LL individuals or from patients suffering from SA.

Interestingly, differences between HL and LL participants were restricted to behavioral and neural responses to negative social feedback, whereas Bayesian analyses revealed evidence for a comparable responsiveness to positive social feedback between groups. Conversely, SA has been consistently found to affect the processing of social rewards (Sripada et al., 2013; Richey et al., 2014, 2017; Schultz et al., 2019). Previous studies point to various negative effects of loneliness on the processing of positive social interactions (Cacioppo et al., 2009; Silva et al., 2017; Lieberz et al., 2021), but findings about the association between loneliness and NAcc reactivity to positive social stimuli are mixed. The involvement of the NAcc in loneliness might be context-dependent, with feelings of social isolation promoting the hedonic experience of positive social stimuli in an acute stage (Tomova et al., 2020), which may be different from chronic loneliness (Saporta et al., 2021). Similarly, lonely individuals might experience a social stimulus as more rewarding only if the stimulus is already familiar (e.g., a romantic partner and not a stranger) (Inagaki et al., 2016). Along these lines, a recent study found no relationship of loneliness with striatal responsiveness to pictures depicting strangers during positive social interactions (D'Agostino et al., 2018). Nevertheless, in our task design, positive feedback was always coupled with monetary gains. Thus, differences regarding positive social feedback might have been obfuscated by the rewarding experience of earning money as evident in enhanced striatal responsiveness to positive feedback, regardless of the partner providing the feedback. Both external (e.g., passive viewing vs being involved in positive social interactions) and internal factors (e.g., state vs chronic feelings of social isolation) may influence the association of loneliness with social reward processing.

Moreover, given the quasi-experimental, cross-sectional design of our study, our findings do not allow casual inferences about the relationship of loneliness and social feedback processing. Additionally, analyses indicate that the observed associations with loneliness were not driven by psychiatric symptoms that were also more pronounced in HL individuals. However, our study specifically focused on high-lonely healthy individuals who may represent a resilient subsample of the population because they did not develop acute psychiatric disorders. Thus, clinical studies with psychiatric patients are warranted to uncover the direction of the observed associative relationships and to further disentangle shared and distinct mechanisms underlying loneliness and psychopathology. Likewise, we cannot exclude the possibility that the LL group may also represent a special, hypersocial group, that differs from the average population. Nevertheless, previous studies indicated that the intensity of loneliness matters mostly for individuals with high loneliness, whereas differences in the experience of loneliness between low and medium lonely individuals had no effect on loneliness-related hypervigilance for social threats (Qualter et al., 2013). While it thus seems unlikely that the inclusion of an intermediate group with average loneliness scores would change the direction of the observed group differences, it might still be of great interest for future studies to investigate clinically relevant cutoff points in either direction. This way, research might help to identify individuals who are at high risk for mental and physical health problems because of high loneliness and in turn to characterize protective mechanisms of highly social individuals that might prevent psychiatric disorders.

Collectively, the current results suggest that loneliness and SA are distinct constructs with specific behavioral and neural substrates. Along these lines, interventions targeting loneliness-specific cognitive biases may be more effective in reducing loneliness than cognitive-behavioral therapies focused on reducing avoidance behavior.

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4. Discussion with references

The studies presented in this thesis aimed at exploring psychosocial, cognitive, and behavioral factors of loneliness and their underlying neurobiological mechanisms. The first study confirmed that alexithymia is an important predictor of loneliness during a social transition from school to university (research aim 1). While loneliness mediated the association of alexithymia with enhanced psychosocial stress, the present results further suggest that a dampened insular reactivity to emotional stimuli may mechanistically underlie the link between alexithymia and loneliness. Importantly, the second study presented in this thesis provided further evidence that loneliness is associated with changes in the AI. The observed loneliness-related phenotype of reduced interpersonal trust and diminished affective and endocrinological reactivity to positive social interactions was associated with impaired activity of the AI during trust decisions. Notably, the diminished functional connectivity of the AI with an occipitoparietal cluster including the precuneus correlated with the attenuated responsiveness to positive social interactions (research aim 2). The results of the current thesis thus extend previous knowledge of loneliness by indicating that an affected insular functioning may serve as an underlying mechanism linking loneliness with both alexithymia and interpersonal trust. Given that the insular cortex is a hub for conscious affect, integrates interoceptive information, and initiates the cognitive processing of salient events (Namkung et al., 2017), these results provide further support for the notion that the ability to shift flexibly between interoceptive and exteroceptive attention is crucial for social connections (Arnold et al., 2019).

Furthermore, loneliness was consistently associated with SA and increased preferred interpersonal distances. However, despite this overlap between loneliness and SA, the results of study 3 indicate that loneliness and SA are separate constructs. By combining frequentist uni- and multivariate analyses with Bayesian statistics, substantial evidence was provided that loneliness is not associated with known correlates of a social-avoidant phenotype (research aim 3). The current thesis thereby expands knowledge of loneliness as a distinct construct. While previous work indicated that the underlying mechanisms of loneliness are distinguishable from those of depression (Shao et al., 2019), the current results add evidence that the neurobiological mechanisms of loneliness are also distinct from those underlying SA. Conclusively, the results of this thesis suggest that established

therapies for related psychiatric disorders cannot be directly applied to reduce loneliness, but interventions should be targeted to the unique mechanisms of loneliness.

4.1. Outlook and limitations

The current thesis hints at alexithymia and impaired interpersonal trust as key factors contributing to the development or maintenance of loneliness. While these factors were chosen due to their crucial role for positive social relationships, as outlined in section 2.1, loneliness is associated with various cognitive biases (Spithoven et al., 2017), and further investigation is required to examine the impact of additional individual components contributing to loneliness. Moreover, further research is needed to allow causal inferences about the relationship between loneliness, alexithymia, and trust. As such, the effectiveness of interventions targeting interpersonal trust or emotional awareness to reduce loneliness should be investigated in randomized controlled trials. In line with the current results, meta-analyses indeed indicated that cognitive-behavioral interventions are more effective in targeting loneliness than social skill trainings (Masi et al., 2011; Zagic et al., 2022), and mindfulness-based interventions to improve present-moment awareness have been found to reduce loneliness (Teoh et al., 2021). However, future studies might examine to what extent an experimental modulation of, for instance, interpersonal trust may augment the therapeutic outcome. An intranasal administration of the neuropeptide oxytocin may be particularly beneficial in this regard, given its positive effects on interpersonal trust and socio-emotional abilities specifically in individuals with high alexithymia and low dispositions to trust (Luminet et al., 2011; Venta et al., 2019). Concurrently, exogenously administered oxytocin might normalize the observed blunted endocrinological responsiveness to social interactions in lonely individuals and address functional attention switching between interoceptive and exteroceptive signals by regulating the diminished AI activity (Yao et al., 2018).

Nevertheless, the question remains which characteristics of lonely individuals might be decisive for the detrimental health effects as the current studies focused on healthy individuals. While this allowed the findings to be attributed to loneliness rather than related psychopathology, the participants of the presented studies might represent a resilient subsample of the population. Nonetheless, the current results confirmed an association of loneliness with enhanced psychosocial stress experiences. This association is in line

with previously observed exaggerated physiological stress responses in lonely individuals (Brown et al., 2018), suggesting that adverse health effects of loneliness might be driven by allostatic load resulting from chronic overactivity of stress systems (McEwen, 1998).
In this context, it would also be of great interest to investigate clinically relevant cutoff points. Previous work indicated that a relationship between loneliness and cognitive biases such as hypersensitivity to social threat is only evident in children suffering from high loneliness (Qualter et al., 2013), but studies that systematically examine at what level of loneliness health problems arise are lacking. Similarly, it is still unclear how long loneliness must persist to affect social cognition or mental and physical health negatively. This is particularly interesting in the light of loneliness serving as a potential mechanism to maintain social homeostasis by signaling a social deficit and initiating adaptive, affiliative behavior (Lee et al., 2021). Obviously, isolating participants for longer periods might be difficult for ethical reasons. However, longitudinal studies involving, for instance, ecological momentary assessments might help to identify the transition point at which potentially prosocial effects of perceived social isolation change for the worse.

4.2. Conclusion

Taken together, the presented studies identified possible starting points for interventions to reduce loneliness by highlighting the significance of alexithymia and reduced interpersonal trust in loneliness. While lonely individuals differed markedly from a social-avoidant phenotype, alexithymic personality traits predicted increased loneliness during periods of social transition, and a compromised neural integration of trust-related information was directly related to attenuated responsiveness to positive social interactions. The findings of the present studies thus offer important implications for the development of scientifically based interventions to target loneliness and provide comprehensive evidence that loneliness is an independent construct, which should be distinguished from depression or SA.

4.3. References

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