A network neuroscience perspective on the neural correlates of state and trait loneliness across the adult lifespan

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Abstract

Cultivating social relationships is a fundamental aspect of being human. Research has shown that various neurocognitive processes support our intrinsic need to connect. A leading theory is that loneliness drives attentional focus externally towards social threats, potentially undermining the ability to form social ties. Consistent with this idea, neuroimaging studies on loneliness have implicated brain regions involved in attention and perceptual processing. However, few studies have examined how interactions between assemblies of functionally connected brain regions, or networks, are altered when experiencing loneliness. Network interactions vary across individuals, are shaped by lived experience, and change with age. Thus, loneliness may impact the brain's network architecture, and these impacts may differ for younger and older adults. In three data-driven studies combining social and cognitive neuroscience with advanced network neuroscience methods, this thesis investigates the impact of loneliness on brain network architecture and changes with age.

Study 1 adopts a state-based approach to investigate the neural correlates of loneliness. We identified converging functional brain activity and co-activation patterns during the experience of loneliness induced by social exclusion using quantitative meta-analytic methods. Results challenge the predominant theoretical account of a shared neural response to social exclusion and physical pain. Findings also highlight the role of the default network is critical for supporting internally-directed cognitive processes, including mentalizing about the thoughts and feelings of oneself and others.

In studies 2 and 3 our focus shifted to the impact of trait loneliness on brain network organization. Here, we investigate whether loneliness is related to differences in the intrinsic functional organization of brain networks that support internally (e.g., mentalizing) and externally (e.g., perception and attention) directed neurocognitive processes. Study 2 establishes a novel whole-brain connectomics approach examining individual differences in resting-state functional connectivity (RSFC). Following previous work, we explore these differences in the context of two other trait variables that impact human sociality: meaning in life (study 2) and empathy (study 3) in a large young adult cohort (mean age = 28.04y, n = 830). Findings from study 2 advance knowledge of the neural correlates of loneliness by revealing greater RSFC between externally-directed (e.g., visual and ventral attention) and association networks (e.g., default and frontoparietal). Loneliness was also related to reduced network modularity, an organizational pattern previously implicated in poorer neurocognitive functioning.

Study 3 explores age differences in RSFC related to loneliness in younger (mean age = 22.6y, n = 128) and older (mean age = 69.0y, n= 92) adults. We show greater visual network integration with association networks in younger adults, replicating study 2. This RSFC pattern was age-invariant in its relationship with empathy. However, loneliness was related to both inter-and intra-network integration of association networks in older adults. Notably, default network regions showed robust associations with loneliness that differed with age, again showing the susceptibility of this network to the experience of loneliness. We consider the implications of these loneliness-related differences among brain regions involved in internally-directed mentation for normal aging and brain disease.

Collectively, these studies advance theories on the neural impacts of state and trait loneliness by providing the first comprehensive investigation of social and brain functioning spanning younger and older adulthood. The findings contribute to a broader understanding of the neural mechanisms underlying loneliness and highlight the utility of network-based approaches for investigating fundamental principles of human sociality.

Résumé

Les liens sociaux sont fondamentales à l'ensemble de la vie sociale humaine. Divers processus neuronaux soutiennent notre désir intrinsèque de se connecter. Selon une théorie dominante, la solitude détourne l'attention vers les menaces sociales dans le monde extérieur, ce qui peut compromettre la formation de liens sociaux. Les études de neuro-imagerie sur la solitude impliquent les régions du cerveau associées à l'attention et à la perception. Cependant, peu d'études examinent comment l'interaction entre ces régions, ou réseaux, est modifié lorsqu'on se sent seul. L'interaction entre les réseaux varient entre individus, en fonction de leurs expériences et changent en fonction de l'âge. Ainsi, la solitude peut avoir un impact sur l'architecture du réseau cérébral qui diffèrent entre les jeunes adultes et les adultes plus âgés. Cette thèse examine l'impact de la solitude sur l'architecture des réseaux cérébraux et les changements de ces réseaux avec l'âge dans le cadre de trois études qui utilisent diverses méthodes avancées des neurosciences sociales et cognitives.

La première étude examine les corrélations neurales de l'état de la solitude. Nous avons identifié une convergence d'activité cérébrale et de coactivation pendant l'état de la solitude induite par l'exclusion sociale en utilisant des méthodes de méta-analyse quantitative. Les résultats mettent en question une théorie dominante qui soutient qu'il existe une réponse cérébrale commune à l'exclusion sociale et à la douleur physique. Les résultats mettent en évidence le rôle du réseau par défaut qui est essentiel au soutien les processus cognitifs dirigés en interne, y compris la mentalisation des pensées et des sentiments de soi-même et des autres.

La deuxième et troisième études examine l'impact de la solitude sur l'organisation des réseaux cérébraux. Ici, nous cherchons à savoir si la solitude est liée à une différence dans l'organisation fonctionnelle intrinsèque des réseaux cérébraux qui soutiennent les processus neurocognitifs internes (la mentalisation) et externes (la perception et l'attention). La deuxième étude établit une nouvelle approche connectomique pour examiner les différences individuelles dans la connectivité fonctionnelle. Les deux études explorent cette différence dans le contexte d'autres variables : le sens de la vie et l'empathie chez les jeunes adultes (âge moyen = 28,04 ans, n = 830). La deuxième étude révèle une augmentation de connectivité entre les réseaux dirigés vers l'extérieur (l'attention visuelle et ventrale) et les réseaux d'association (les réseaux par défaut et frontopariétaux). La solitude était également liée à une réduction de modularité neurale, un modèle d'organisation précédemment impliqué a la réduction du fonctionnement neurocognitif.

La troisième étude explore les différences de connectivité liées à la solitude entre les adultes plus jeunes (âge moyen = 22,6 ans, n=128) et plus âgés (âge moyen = 69,0 ans, n=92). Nous montrons une plus grande intégration du réseau visuel avec les réseaux d'association chez les jeunes adultes, reproduisant les résultantes de la deuxième étude. La connectivité était invariable selon l'âge dans sa relation avec l'empathie. Cependant, la solitude était liée à l'intégration inter- et intra-réseau des réseaux d'association chez les adultes plus âgés. Notamment, les régions du réseau par défaut ont montré des associations robustes avec la solitude qui différaient avec l'âge, montrant à nouveau l'importance de ce réseau à l'expérience de la solitude.

Collectivement, ces études progressent les théories sur l'impacts neuronaux de la solitude en fournissant la première enquête complète sur le fonctionnement social et cérébral entre les jeunes et les adultes plus âgés. Les résultats contribuent à une meilleure compréhension des mécanismes neuronaux qui support la solitude et soulignent l'utilité des approches basées sur les réseaux pour étudier les principes fondamentaux de la socialité humaine.

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To Jabaree—without you, I would not have ”surpassed my limits!”.

"Lying, thinking

Last night

How to find my soul a home

Where water is not thirsty

And bread loaf is not stone

I came up with one thing

And I don't believe I'm wrong

That nobody,

But nobody

Can make it out here alone.

Alone, all alone

Nobody, but nobody

Can make it out here alone."

– Maya Angelou

Excerpt from *"Alone"*

Contribution to Original Knowledge

The work presented in this thesis provides original scholarship aimed at addressing current gaps in our understanding of how *state* and *trait* loneliness impacts brain function across adulthood. By employing advanced network neuroscience methods and multivariate analytic techniques, this work builds a deeper and broader perspective on the neural mechanisms underlying the experience of loneliness. Through three studies, this work provides novel insights into how brain functional organization reflects individual and age-related differences in loneliness and how these neural differences relate to core principles that drive and support human sociality. Summarized below are the main contributions made by each study:

Chapter 2

- Using data-driven meta-analytic methods we found that the neural response to social exclusion evoked using the Cyberball task — one of the most common paradigms for studying social exclusion — does not reliably activate the dorsal anterior cingulate. This region of the brain is reliably involved in the experience of physical pain and more recently has been associated with feelings of social exclusion or "social pain".

- Instead, social exclusion reliably engages the default network, a functionally connected assembly of brain regions associated with social and self-referential cognitive processes.

- These findings directly question a prominent social cognitive theory that posits a shared neural system underlying the experience of physical pain and the affective response to social exclusion.

Chapter 3

- Using a whole-brain connectome approach, we demonstrated that individual differences in whole-brain resting-state functional connectivity corresponded to individual differences in *trait* loneliness and meaning in life.
- Greater self-reported loneliness was associated with dense connections between default network with externally-directed perceptual networks.
- Individuals with a high levels of loneliness reported finding life less meaningful, and the relationship between these two trait variables were dissociable at the level of brain.
- Greater self-reported meaning in life was associated with increased connectivity between default and limbic networks.
- Loneliness is associated with lower modularity (i.e., increased integration) brain network organization, whereas meaning in life is associated with higher modular network (i.e., increased segregation).

Chapter 4

- Age differences in the relationship between loneliness *trait*, dimensions of empathy functioning (i.e., emotional recognition and aspects of empathy of traits) and functional organization of the brain network were observed.
- *Trait* loneliness in younger adults was related to greater integration of the visual network and higher order association networks. This pattern of RSFC was age-invariant in its relationship with empathic functioning across groups.

- The association between *trait* loneliness and RSFC differed between younger and older adults. Greater integration among higher-order association networks was related to higher levels of reported loneliness.
- Different dimensions of empathic functioning varied with age. Emotional recognition was characterized by greater intra- and inter-network connectivity of association networks in both age groups. In contrast, self-reported trait empathy measures in younger adults were associated with greater visual network connectivity with the rest of the brain.
- Based on these age differences in associations between social function and RSFC, we suggest that with advancing age the experience of loneliness may engage more internally-oriented versus externally-oriented processing regions.

Contribution of Authors

This thesis integrates original work in Chapters 2, 3, 4, for which I am the lead author. My role in all three studies involved study conceptualization and design, data processing, data analysis, method implementation, interpreting the results, and drafting of the manuscripts. However, my work is, by nature, interdisciplinary and would not be possible without all the talented co-authors whose input and support was indispensable to this body of work. Below I list each of their contributions:

Chapter 2

- R. Nathan Spreng: study conceptualization, interpretation of findings, manuscript writing, overall supervision

Chapter 3

- Tian Ge: data processing, quality assessment
- Minqi Chong: development of the Group Prior Individual Parcellation code,data processing, quality assessment
- Michael A. Ferguson: study conceptualization, interpretation of results
- Bratislav Misic: methodological consultation, interpretation of results
- Anthony L. Burrow: interpretation of results
- Richard M. Leahy: development of the Group Prior Individual Parcellation pipeline

- R. Nathan Spreng: study conceptualization, interpretation of findings, manuscript writing, overall supervision
- All co-authors: manuscript review and editing

Chapter 4

- Roni Setton: data curation, multi-echo fMRI data processing
- Danilo Bzdok: manuscript editing
- Gary Turner: data acquisition
- R. Nathan Spreng: study conceptualization, interpretation of findings, manuscript writing, overall supervision
- All co-authors: manuscript review and editing

All contributions made during the PhD

For completeness, listed below is a full list of all first and co-authored publications from my doctoral training. In the different publications, my main contributions included processing individualized parcellations of resting-state fMRI data as well as preprocessing, analyzing and interpretation of neuroimaging data. I provided intellectual contributions to all projects and helped revise and edit all manuscripts.

* Equal contribution

- **Mwilambwe-Tshilobo, L.**, Setton, R.,Bzdok, D., Turner, G. R., & Spreng, R. N. (*in press*). Feeling alone and feelings for others: Age differences in functional brain networks associated with loneliness and empathy.
  Included in Chapter 4

- Kantarovich, K., **Mwilambwe-Tshilobo, L.**, Fernández-Cabello, S., Setton, R., Baracchini, G., Lockrow, A. W., Spreng, R. N., & Turner, G. R. (2022). White matter lesion load is associated with lower within- and greater between- network connectivity across older age. Neurobiology of aging, 112, 170–180.
  https://doi.org/10.1016/j.neurobiolaging.2022.01.005

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  https://doi.org/10.1038/s41597-022-01231-7

- Setton*, R., **Mwilambwe-Tshilobo*, L.**, Girn, M., Lockrow, A. W., Baracchini, G., Hughes, C., Lowe, A. J., Cassidy, B. N., Li, J., Luh, W. M., Bzdok, D., Leahy, R. M., Ge, T., Margulies, D. S., Misic, B., Bernhardt, B. C., Stevens, W. D., De Brigard, F., Kundu, P., Turner, G. R., & Spreng, R. N. (2022). Age differences in the functional architecture of the human brain. Cerebral cortex, bhac056.
  https://doi.org/10.1093/cercor/bhac056

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  Included in Chapter 2

- Spreng, R. N., Dimas, E., **Mwilambwe-Tshilobo, L.**, Dagher, A., Koellinger, P., Nave, G., Ong, A., Kernbach, J. M., Wiecki, T. V., Ge, T., Li, Y., Holmes, A. J., Yeo, B., Turner, G. R., Dunbar, R., & Bzdok, D. (2020). The default network of the human brain is associated with perceived social isolation. Nature communications, 11(1), 6393. https://doi.org/10.1038/s41467-020-20039-w

- **Mwilambwe-Tshilobo, L.**, Ge, T., Chong, M., Ferguson, M. A., Misic, B., Burrow, A. L., Leahy, R. M., & Spreng, R. N. (2019). Loneliness and meaning in life are reflected in the intrinsic network architecture of the brain. Social cognitive and affective neuroscience, 14(4), 423–433. https://doi.org/10.1093/scan/nsz021
  Included in Chapter 3

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Chapter 1

Introduction

1.1 General context and objectives

1.1.1 General context

In January 2018, the United Kingdom appointed the world's first minister of loneliness in response to growing concerns about public health-related issues related to the rising rates of loneliness and isolation. These concerns stemmed from mounting empirical evidence documenting the adverse effects of loneliness on mental and physical health (Cacioppo & Patrick, 2008; Tilvis et al., 2011, Shankar et al., 2013, d'Oleire Uquillas et al., 2018), particularly among older adults (for review see Ong et al., 2016). The global impact of the COVID-19 pandemic on human sociality further substantiated these concerns internationally. The physical distancing and isolation measures put in place to mitigate the spread of the virus inadvertently left many people worldwide feeling socially isolated and yearning for social connection.

Human sociality is rooted in a fundamental need for social connection. While people might be intrinsically motivated to form and maintain social bonds, our social needs are sometimes left unmet, and feelings of loneliness emerge (Cacioppo & Hawkley, 2009). Decades of social and cognitive neuroscience research have led to integrated theories on

the emergence of human social cognitive processes (e.g., Tomasello, 1999) and the brain systems that support human social functioning (Dunbar, 2003; Adolphs, 2009). They have shown that a range of neurocognitive processes supports our ability to navigate complex social environments and interact with others through synchronized activity among spatially distributed brain regions. However, our understanding of how loneliness impacts the interactions among large-scale brain networks and the implications these changes have on social functioning across the lifespan remains poorly understood.

Converging neuroimaging evidence has demonstrated the existence of a "lonely brain," characterized by altered functional and structural changes in multiple brain regions (Cacioppo et al., 2009, Kanai et al., 2015; Lan et al., 2016; Inagaki et al., 2016; Courtney & Meyers, 2020; Spreng et al., 2020; Lam et al., 2021). The experience of social isolation is theorized to influence perceptual and attentional processes resulting in hypervigilance toward social threats (Cacioppo et al., 2009; Cacioppo et al., 2018). The effects of loneliness on neurocognitive processes are mirrored by changes in the organization of functional brain networks including diminished coupling of cognitive control networks with lower-level visual networks that may be involved in stimulus-driven social perception (Layden et al., 2017; Tian et al., 2017; Feng et al., 2019). Previous neuroimaging studies have identified brain regions and neurocognitive processes that are altered in lonely individuals. However, we are aware of no previous studies that have examined these interactions in the context of individual differences in functional brain network organization (Wang et al., 2015, Kong et al., 2019) and changes with age (Chan et al., 2014; Geerligs et al., 2015; Setton & Mwilambwe-Tshilobo et al., 2022). A key question then remains: How do individual differences and age-related changes in the network organization of the brain influence the relationship between loneliness and brain function?

1.1.2 Objectives

The primary objective of the current thesis is to provide an integrative framework to characterize the relationship between loneliness and brain network functional organization

across the adult lifespan. Neuroimaging studies have been integral for identifying brain regions underlying loneliness. However, it is also important to understand how the interactions and organization of these regions are influenced by the experience of loneliness, and how they relate to factors that impact human sociality. Furthermore, because studies investigating the neural bases of loneliness have predominantly focused on young adult populations, little is known about how differences in brain function associated with loneliness change as people age. Filling this gap is critical as it may reveal basic neural mechanisms by which loneliness might affect brain function and potential implications for neurocognitive aging in late-adulthood.

Chapter 1

The present thesis aims to expand upon previous work on the neural correlates of state and trait loneliness by examining brain network functional organization in younger and older adults. The objective of Chapter 1 is to introduce loneliness, synthesize the relevant and current neuroimaging studies and theoretical accounts of the neural basis of loneliness, and highlight how leveraging approaches from network neuroscience might help address open questions on the relationship between loneliness and brain function.

Chapter 2

Loneliness is multi-dimensional, and can be experienced as a momentary feeling induced bound to a specific social context (*state* loneliness). Chapter 2 presents a quantitative meta-analytic review of the neuroimaging literature on *state* loneliness, focusing specifically on the neural response to social exclusion. The objective of Chapter 2 is to use a data-driven approach to identify patterns of brain activity and co-activation patterns reliably engaged by the *experience* of social exclusion and evaluate these patterns with respect to the leading hypothesis on associated neural mechanisms.

Chapter 3

Feelings of loneliness can also be experienced more broadly, irrespective of social context (*trait* loneliness). Contemporary neural accounts of *trait* loneliness suggest perceptual, attentional, and affective processes play an important role in shaping an individual's experience of loneliness. The objective of Chapter 3 was to investigate these neural accounts using resting-state fMRI by examining individual differences in the relationship between *trait* loneliness and whole-brain functional connectivity. Additionally, this study offers a methodological innovation by providing a novel framework that interrogates individual differences in the intrinsic functional organization of the brain across multiple topological scales (inter-regional and network-level).

Chapter 4

The primary objective of Chapter 4 is to use this framework to address open questions related to disparate findings in the functional connectivity patterns associated with two core dimensions of social functioning: *trait* loneliness and empathic responding. First, we address a contradiction in reported associations between *trait* loneliness and brain function in early and middle-aged adults by expanding our studies to a healthy aging sample, thereby providing a full lifespan account of the "lonely brain." A secondary objective of chapter four is to examine individual and age-related differences in the patterns of connectivity in association with a second key feature of sociality: Empathic functioning.

Chapter 5

Finally, the objective of Chapter 5 is to summarize the studies included in this thesis and describe the contribution of the work to the field of social neuroscience.

1.2 Literature review

1.2.1 Defining loneliness: a multidimensional construct

To provide guidance for the reader and delineate the scope of the current thesis, it is important to note that loneliness can be conceptualized as either a *state* or a *trait*. State loneliness is dependent on a social context. For example, feeling lonely when transitioning to college away from family and friends for the first time or being excluded by others during a specific social context (e.g., being discriminated against in the workplace). *Trait* loneliness is dispositional, and feelings of isolation remain regardless of the social situation one might find themselves in. Most neuroimaging studies typically examine loneliness as a trait through questionnaires, the most commonly used being the UCLA loneliness scale (Russell et al., 1980). *State* loneliness has mainly been considered in neuroimaging studies from a social pain perspective (Eisenberger et al., 2003). This perspective has been taken to support the theory that loneliness is an aversive state that serves as a biological signal (similar to thirst or hunger) to motivate individuals to attend externally to others to mend or create new bonds (Cacioppo & Patrick, 2008; Cacioppo et al., 2018). As part of the brain's response to perceived social isolation, it triggers implicit changes that increase short-term self-preservation by facilitating the detection and response to social threats, which, in the long run, can have deleterious effects (Cacioppo et al., 2018). Therefore, studies examining social exclusion or rejection by others offer a window into the brain's response to state social isolation. Unless expressly stated, the studies summarized in the subsequent sections and the target of studies in Chapters 3 and 4 focus on trait loneliness.

Loneliness is a common human emotion; however, it is also a complex and unique experience for each individual. Loneliness is related to the quantity (number of social contacts) and the quality of people's social relationships (Perlman & Peplau, 1981). From a theoretical perspective, each person's relationships must adequately satisfy an inherent set of social needs which, if unmet, can be distressing (Perlman & Peplau, 1981). However, each person has a different threshold for the level of social interaction they need. For

instance, a person who is socially isolated may not necessarily feel lonely, while someone else may have many social relationships yet still feel lonely. Loneliness is not strongly correlated with the amount of time spent alone (Hawkley, Burleson, Berntson, & Cacioppo, 2003), size of a social network, or frequency of contact (Luhmann & Hawkley, 2016), but rather by how people feel about each of these objective social measures. At its core, loneliness is the *subjective perception* of social isolation. In the next section, we briefly review why characterizing the neural determinants and impact of loneliness are emerging as a critical public health issue. In the following sections, we focus on what is currently known about the "lonely brain" and describe how the studies that comprise this dissertation fill critical knowledge gaps in this area and the emerging field of social network neuroscience more broadly.

1.2.2 The impact of loneliness on health and well-being across adulthood

Previous studies suggest that the distribution of loneliness across adulthood is U-shaped —with higher rates of loneliness in early adulthood (<30 years) and late adulthood (>80 years) (Lasgaard, Friis, & Shevlin, 2016; Luhmann & Hawkley, 2016). Other studies, however, report a higher prevalence of loneliness in young adult populations (D'Agostino et al., 2018) or find no relationship between age and loneliness (Hawkley & Cacioppo, 2007). While it is unclear whether loneliness is as prevalent or more prevalent among younger or older adults, life transitions seem to contribute to the peaks. For example, isolation is a risk factor for loneliness among older adults because living alone reduces social contact and increases the likelihood of experiencing more social isolation (Luhmann & Hawkley, 2016). While younger and middle-aged adults also experience critical life transitions (i.e., college, marriage/divorce) that may cause them to feel isolated from their social network, older adults disproportionally experience an accumulation of risk factors that contribute to loneliness (Luhmann & Hawkley, 2016). Risk factors such as

loss of spouse (Pinquart, 2003), retirement, and the absence of high-quality social relationships (Luhmann & Hawkley, 2016) can independently contribute to loneliness. However, because these factors are often compounded in late adulthood, loneliness may be particularly detrimental in this population segment.

Extensive research has also documented loneliness's direct and indirect impact on health outcomes. Loneliness is a risk factor for depression and anxiety (Cacioppo et al., 2006; Holvast et al., 2015), hypertension, reduced physical activity, and insomnia (Shankar, McMunn, Banks, & Steptoe, 2011; Ong, Uchino, & Wethington, 2016). In addition, in a study of 2,173 community-living healthy (non-demented) older adults from the Amsterdam Study of the Elderly, lonely individuals were 1.64 times more likely to develop Alzheimer's disease over three years (Holwerda et al., 2014). Many of these health-related issues are cumulative and create a negative trajectory of events that increase older adults' mortality risk (Hawkley & Cacioppo, 2003; Tilvis et al., 2004). However, loneliness can also negatively influence health through indirect routes. For instance, meaning in life is a critical component of subjective wellbeing that can be defined as the sense that one's life has purpose, significance, and coherence (Martela & Steger, 2016). Meaning in life is an important protective factor related to various health outcomes, longevity, reduced morbidity, and social engagement (Steptoe & Fancourt, 2019). Yet, lonely people are more likely to evaluate their life as less meaningful than those with rewarding interpersonal relationships (Stillman et al., 2009), thereby depriving them from this source of wellbeing. Therefore, these studies demonstrate that loneliness is not just an aversive feeling but has a wide-ranging implications for health and wellbeing.

1.2.3 The lonely brain: Insights from neuroimaging studies

Loneliness can be adaptive and motivate individuals to reconnect. However, it can also lead to maladaptive changes in cognition that heighten social vigilance (Bangee, Harris, Bridges, Rotenberg, & Qualter, 2014), biasing attention and memory for negative social information (Gardner, Pickett, Jefferis, & Knowles, 2005; Cacioppo, Balogh, & Cacioppo,

2015; Bangee & Qualter, 2018). Collectively, these changes impact how people process information from their immediate social environment. According to the Evolutionary Theory of Loneliness, the aversive feeling of loneliness activates behavioral and cognitive processes, allowing people to avoid negative social situations and facilitating opportunities to reinstate social bonds (Cacioppo & Hawkley, 2009; Cacioppo & Cacioppo, 2018). Although the short-term effects serve an adaptive function in promoting social reengagement, loneliness can also trigger a maladaptive regulatory loop of cognitive processes and behaviors that reinforces the perception of social isolation. The consequences of these selective changes in cognition may further reinforce a negative perceptual view of the social world, making it harder to reconnect with others (Cacioppo & Cacioppo, 2018). Thus, feelings of loneliness can have both adaptive and maladaptive consequences on human social cognition and behavior. This theoretical account has been generally supported by neuroimaging research examining the impacts of loneliness on brain structure and function. Here, key findings from this growing body of work are highlighted.

Impact of loneliness on brain structure in adulthood.

Although loneliness is associated with a distinct cognitive and behavioral profile, the neural mechanisms underlying these changes are still poorly understood. Neuroimaging studies have shed some light by linking loneliness with structural changes in the brain (Kanai et al., 2012; Spreng et al., 2020) and have provided converging evidence implicating the default network in the experience of loneliness. The default network is a large-scale brain network with extensive spatial overlap with the 'social brain'— an ensemble of brain regions involved in social cognition (Mars et al., 2012). It is implicated in various social and self-reflective processes, such as inferring others' beliefs and intentions, perceiving and interpreting others' emotions, and autobiographical memory (Andrews-Hanna, 2012). Lonely young adults show less grey matter volume in the left posterior superior temporal sulcus, a region implicated in social perception (Kanai et al., 2012). Reduced white matter density in this region was also found in addition to multiple other regions

in the default network, such as bilateral inferior parietal lobule, dorsal medial prefrontal cortex, and the temporoparietal junction (Nakagawa et al., 2015). More recently, a study conducted in a middle-aged adult cohort (age range: age range 40-69) found that grey matter volume in multiple regions of default (e.g., posterior superior temporal sulcus, left temporoparietal junction, dorsal anterior cingulate cortex) was positively associated with loneliness (Spreng et al., 2020).

In contrast, a study conducted on older adults (age range: 61-81) found no differences in gray matter volume in regions of the default network but in areas implicated in emotional regulation and cognitive processing (Düzel et al., 2019). These changes in brain structure suggest that loneliness affects regions involved in social cognition across young and middle-aged adults. However, they also indicate that loneliness's impact on brain structure may vary with age.

Impact of loneliness on brain function in adulthood

Brain structural architecture determines but does not entirely restrict the functional dynamics of the brain (Honey et al., 2007; Suárez et al., 2020). Several approaches can be utilized to examine brain function, but only relevant studies using functional magnetic resonance imaging (fMRI) are considered for this thesis. Early work using the task-fMRI paradigm to investigate loneliness and brain function has provided insight into possible mechanisms by which loneliness might contribute to changes in social and affective processes (Cacioppo et al., 2009; Inagaki et al., 2016). Most notable was the first task-fMRI study on loneliness, which compared the neural response of lonely and non-lonely young adults as they were shown pictures of social and non-social stimuli that varied in emotional valence (positive, neutral, negative; Cacioppo et al., 2009). They found that lonely young adults showed a dampened activation within brain regions implicated in reward processing (e.g., ventral striatum) to positive social (vs. positive non-social) images. Lonely participants also showed decreased activation in the temporoparietal junction when viewing negative social (vs. negative non-social) images, which was in-

terpreted as lonely individuals being less likely to engage in social cognitive processes that require taking on the perspective of others. In contrast, lonely participants showed greater activation in the visual cortex in response to negative social (vs. negative non-social) images (Cacioppo et al., 2009). This latter finding has been interpreted as evidence of implicit hypervigilance to social threats and empirical support for the theoretical account of loneliness.

To explore how individual differences in loneliness might relate to brain function, other researchers have used resting-state fMRI (rs-fMRI) to examine individual differences in the intrinsic functional connectivity of the brain. Without using explicit task demands, rs-fMRI explores the spontaneous neural activity of the brain (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995). This pattern of intrinsic neural activity between brain regions reflects patterns of co-activation observed during task-based fMRI studies. Specifically, the co-activation of these regions that are simultaneously engaged during the performance of a task fluctuate together during rs-fMRI and are consistent within an individual over time, and can therefore serve as a sensitive measure to predict individual differences in behavior and cognition (Stevens & Spreng, 2014).

A few studies have used rs-fMRI to investigate the relationship between loneliness and interactions in resting-state brain networks in young adults (Tian et al., 2017; Layden et al., 2017; Feng et al., 2019). For instance, Layden et al. (2017) reported that loneliness was related to altered resting-state functional connectivity among two neural networks associated with attentional processes. These included the cingulo-opercular network, commonly related to sustained maintenance of task control and task goals, and the frontoparietal network, which supports adaptive control of attention (Dosenbach et al., 2007). Using a rs-fMRI functional connectivity analytic approach, they demonstrated that loneliness was associated with increased within-network functional connectivity of the cingulo-opercular network and reduced connectivity between the cingulo-opercular and the frontoparietal network (Layden et al., 2017). These findings suggest that loneliness is characterized by changes in connectivity strength within and between networks that sup-

port the maintenance and flexible control of attention. More importantly, these findings point to a putative mechanism whereby diminished top-down control of attention may contribute to the implicit hypervigilance to social threats that characterize loneliness.

The majority of studies using rs-fMRI have been conducted in younger adults. Spreng et al. (2020) examined whole-brain resting-state functional connectivity in a cohort of middle-aged adults using data from the UK Biobank. This study takes a slightly different approach than Layden et al. (2017) in that it uses a pre-define cortical atlas to divide the cerebral cortex into discrete brain regions, each belonging to one of 7 large-scale canonical networks (Yeo et al., 2011). This study showed that participants with higher self-reported loneliness had greater resting-state functional connectivity within the default network. In addition, lonely participants showed higher integration of the default with frontoparietal and limbic networks (Spreng et al., 2020). Critically, the default network was negatively correlated with the visual network. These findings, along with other findings associating loneliness with changes in default networks integrity, were interpreted as evidence that loneliness in middle age may involve more internally-directed cognitive processes.

Implications of aging on the relationship between loneliness and brain function

While the research conducted in younger adults seems to support the theoretical account that loneliness involves brain regions and networks that support externally-directed processes, findings from the few studies conducted with middle-aged or older adults are not entirely consistent with this perspective (Wong et al., 2016; D'Agostino et al., 2018; Düzel et al., 2019; Spreng et al., 2020). What seems consistent across age groups is that the default network appears to be involved in the experience of loneliness. However, loneliness-default network associations may differ by age. Findings from two studies exemplify why the current neural account of loneliness may need to be modified to account for how aging might alter the impact of loneliness on the brain.

One examined the relationship between individual differences in loneliness and resting-state functional connectivity (Lan et al., 2016). They reported that increased functional

connectivity within the lingual gyrus—a region within the default network involved in social cognition and processing—was positively correlated with loneliness. While this finding is consistent with the notion that loneliness alters connectivity within the default network, unlike previous rs-fMRI studies conducted in young adults (Layden et al., 2015), no association was found between loneliness and regions of the brain involved in attention or executive control. Similarly, a recent multimodal neuroimaging study in middle-aged adults found that associations with loneliness converged on the default network across multiple scales (grey matter morphology, white-matter microstructure, and rs-fMRI data) across multiple scales (Spreng et al., 2020). Notably, the results looking at whole-brain RSFC showed greater connectivity within the default network and greater integration between the default and other higher-order association networks.

These findings suggest that loneliness is associated with changes in networks that support internally-directed neurocognitive processes in older adults. But it is difficult to test this hypothesis without directly comparing young and older adults. Part of the difficulty in addressing this gap in the literature is that few studies have been conducted on older adults. Several studies have demonstrated that aging is associated with weaker connectivity within resting-state networks (Chan et al., 2014; Geerligs et al., 2015; Wig, 2017; Setton & Mwilambwe-Tshilobo et al., 2022). Weaker intrinsic connectivity may reflect relative dysfunction of the modular organization (where connectivity within a network is strong, but between network connectivity is weak), which is a fundamental organizing principle of the brain (Sporns & Betzel, 2016). Could these age-related differences in brain functional organization contribute to why loneliness might differentially impact the brain with age? If so, what might be the implications of these neural differences for sociality at different stages of adulthood?

1.2.4 The current research

Loneliness impacts multiple neurocognitive processes and alters the functional integrity of the networks that support these processes. Although multiple studies have investig-

ated brain regions associated with loneliness, fewer studies have examined the functional interactions among them and how they change with age. In the studies that comprise this dissertation, we postulate that loneliness is a complex construct encompassing multiple neurocognitive processes. As such, examining the interactions within and between distinct brain networks may provide a more comprehensive perspective of the neural account of this complex social construct. Network neuroscience offers an array of tools to characterize the functional organization of the human brain (Bassette & Sporns, 2017). Such approaches have been readily implemented to characterize complex brain networks in various contexts, such as aging (Chan et al., 2014; Geerligs et al., 2015; Hughes et al., 2020; Setton & Mwilambwe-Tshilobo et al., 2022), real-world social network structure (Hyon et al., 2020; Baek et al., 2022), or during social interactions (Schmälzle et al., 2016). The current thesis combines social and cognitive neuroscience with network neuroscience methods across three data-driven studies (one on state loneliness and two on trait loneliness) to advance our understanding of individual and age-related differences in the impact of loneliness on the functional network architecture of the human brain.

Chapter 2

Social exclusion reliably engages the default network: A meta-analysis of Cyberball

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2.1 Preface

In this chapter, we consider loneliness as a state — a momentary experience of social isolation bound to a specific social context. Here, the particular context is the experience of social exclusion by others. A predominant theory in the field of social neuroscience is that the neural response to social exclusion or rejection (social pain) overlaps with that of physical pain in two key regions: the dorsal anterior cingulate (dACC) and the anterior insula (AI). However, the involvement of these two key regions has not always been reproducible, rendering the theory of social pain a topic of much debate. A commonly used method to estimate the convergence of neural activity across multiple independent

neuroimaging studies is coordinate-based meta-analysis. When this study was published, only two studies used this data-driven method to identify reliable brain regions activated during social exclusion across neuroimaging studies. However, no meta-analytic study had leveraged coordinate-based meta-analytic methods to characterize the functional networks underlying the neural reactivity to social exclusion across neuroimaging studies. Implementing such an approach would provide an unbiased account that does not constrain the brain's response to social exclusion to individual brain regions but rather models the underlying functional networks involved. Therefore, the goal of the current study was to conduct a quantitative meta-analysis to identify brain regions reliably activated during the social exclusion and co-activation patterns involved in this experience of state loneliness.

2.2 Abstract

Social exclusion refers to the experience of being disregarded or rejected by others and has wide-ranging negative consequences for well-being and cognition. Cyberball, a game where a ball is virtually tossed between players, then leads to the exclusion of the research participant, is a common method used to examine the experience of social exclusion. The neural correlates of social exclusion remain a topic of debate, particularly with regards to the role of the dorsal anterior cingulate cortex (dACC) and the concept of social pain. Here we conducted a quantitative meta-analysis using activation likelihood estimation (ALE) to identify brain activity reliably engaged by social exclusion during Cyberball task performance (Studies = 53; total N = 1,817 participants). Results revealed consistent recruitment in ventral anterior cingulate and posterior cingulate cortex, inferior and superior frontal gyri, posterior insula, and occipital pole. No reliable activity was observed in dACC. Using a probabilistic atlas to define dACC, fewer than 15% of studies reported peak coordinates in dACC. Meta-analytic connectivity mapping suggests patterns of co-activation are consistent with the topography of the default network. Reverse infer-

ence for cognition associated with reliable Cyberball activity computed in Neurosynth revealed social exclusion to be associated with cognitive terms Social, Autobiographical, Mental States, and Theory of Mind. Taken together, these findings highlight the role of the default network in social exclusion and warns against interpretations of the dACC as a key region involved in the experience of social exclusion in humans.

2.3 Introduction

Exclusion from social participation is an all too common, yet psychologically painful, facet of the human experience. Being bullied by peers at school, discrimination at the workplace, and rejection from a romantic partner are all experiences that can lead a person to feel the sting of social exclusion. This sensitivity to social exclusion is deeply rooted in a need for social connectedness (Baumeister & Leary, 1995; Williams et al., 2000). Consequently, the brain has developed systems to efficiently recognize and respond to signs of social exclusion across a range of situations (J. Cacioppo & Hawkley, 2009; Eisenberger et al., 2003; Fisher et al., 2010; Masten et al., 2011). Due to its pervasiveness and importance for human functioning, social neuroscientists have sought to understand the underlying neural processes involved in reactions to social exclusion.

Previous neuroimaging studies have examined the neural correlates of social exclusion. These studies vary in their approach, but one of the most commonly employed paradigms used to evoke feelings of social exclusions in an experimental setting is the Cyberball task. Cyberball is a computerized virtual ball-tossing game played between the participant and other virtual players (Williams et al., 2000). The traditional Cyberball paradigm involves two rounds: an "inclusion" round during which the ball is received and tossed equally among all players, subsequently followed by an "exclusion" round during which the other players no longer pass the ball to the participant, thereby eliciting feelings of social exclusion. In their seminal study, Eisenberger et al. (2003) used a Cyberball task to investigate the neural response to social exclusion. Results from this

study showed increased activity in the dorsal anterior cingulate (dACC), anterior insula, and right ventral prefrontal cortex during the exclusion round relative to the inclusion round (Eisenberger et al., 2003). Critically, increased activity in the dACC and anterior insula were shown to correlate with self-reports of social distress after exclusion. Based on prior work demonstrating activity of the dACC during the experience of physical pain (Rainville et al., 1997; Singer et al., 2004), this finding was interpreted to suggest that social exclusion is experienced as 'painful' and led to the hypothesis of overlap in the neural circuitry underlying social pain and physical pain (Eisenberger, 2012a, 2012b; Eisenberger et al., 2003; Lieberman & Eisenberger, 2015).

Subsequent studies have since substantiated this claim. Activation of the dACC has been reported during Cyberball (Dewall et al., 2010; 'I feel your pain: emotional closeness modulates neural responses to empathically experienced rejection', 2011; Lieberman & Eisenberger, 2015; Onoda et al., 2010) and other social exclusion paradigms (O'Connor et al., 2008; Sebastian et al., 2011). Similar findings have also been observed during third person ('I feel your pain: emotional closeness modulates neural responses to empathically experienced rejection', 2011; Meyer et al., 2013) or recollected experiences of social exclusion (Kross et al., 2011). However, findings from several studies suggest that the dACC is not specific to the experience of social or physical pain, but instead responds to various cognitive and emotional events (Kragel et al., 2018; Perini et al., 2018; Somerville et al., 2006; Wager et al., 2016). Other studies have shown that the emotional responses to social exclusion involves the subgenual subdivision of the anterior cingulate cortex rather than the dACC (Bolling, Pitskel, Deen, Crowley, McPartland et al., 2011; Masten et al., 2009), hinting at dissociable neural representations for physical and social pain (Woo et al., 2014). Thus, while the dACC has been highlighted as key region within the literature, the lack of consistent correspondence between the neural correlates of social and physical pain have led to questions regarding the association between social exclusion and dACC.

Earlier meta-analyses of functional imaging studies aimed at identifying reliable neural correlates of social exclusion have also provided inconclusive results. When restrict-

ing the analysis to the anterior cingulate, one meta-analysis showed involvement of the dACC during social exclusion (Rotge et al., 2015). Yet, when examining across studies of social exclusion, irrespective of dACC reported activity, other meta-analytic studies have failed to find reliable dACC activity (S. Cacioppo et al., 2013; Vijayakumar et al., 2017). Moreover, when focusing specifically on neuroimaging studies of social exclusion using the Cyberball task, the dACC did not emerge as a region that was reliably engaged across 29 studies (Vijayakumar et al., 2017). In contrast, more ventral anterior cingulate cortex (vACC) as well as ventral prefrontal cortex and orbitofrontal cortex were more reliably recruited across past meta-analyses (S. Cacioppo et al., 2013; Vijayakumar et al., 2017). Further, regions of the default network have also been implicated in mentalizing about the intentions of other people, both during (Bolling, Pitskel, Deen, Crowley, Mayes et al., 2011; Onoda et al., 2010; Wagels et al., 2017) and after (Powers et al., 2013) social exclusion. Therefore, engagement of this network may constitute an important component of the intrapersonal and interpersonal processes of social exclusion (Kawamoto et al., 2015). However, the extent to which the default network is engaged in social exclusion requires further investigation.

The present study aims to identify areas of convergence in functional activity and co-activation patterns of brain regions engaged during social exclusion measured during Cyberball. Using coordinate-based activation likelihood estimation (ALE) meta-analysis (Eickhoff et al., 2012; Eickhoff et al., 2009), we identify reliable whole-brain activation patterns of social exclusion across neuroimaging studies using traditional and alternating (interspersed sequences of inclusion and exclusion) Cyberball designs. This study extends prior meta-analyses on social exclusion (S. Cacioppo et al., 2013; Vijayakumar et al., 2017) in several ways: First, we use meta-analytic connectivity modeling (MACM) (Eickhoff et al., 2011; Laird et al., 2009) to characterize the functional connectivity profile of regions identified in the ALE analysis. Second, we use Neurosynth (Yarkoni et al., 2011) to meta-analytically decode the cognitive processes associated with the identified neural patterns from the ALE analysis. Finally, we directly investigate whether dACC, a

core node in the hypothesized common substrate of physical and social pain, is reliably engaged by Cyberball. Taking this approach allows us to not only delineate brain regions that have consistently been associated with social exclusion, but it can provide new insights into putative neural networks associated with social exclusion, and decode the psychological processes of these brain regions using valid reverse inference, in the largest sample of studies currently available.

2.4 Methods

2.4.1 Literature search and study selection

We performed a systematic review of functional magnetic resonance imaging studies investigating the neural correlates of social exclusion using Cyberball. All articles in the literature published from October 10th, 2003 to August 19th, 2020 were considered for this meta-analysis. We used PubMed/MEDLINE, and PsychINFO online databases to search for articles with abstracts, titles, and keywords using the following search string: (social rejection OR social exclusion OR ostracism) AND (MRI OR fMRI OR functional magnetic resonance imaging OR brain imaging). The search yielded 341 articles. Reference lists of relevant articles were manually searched for additional publications not captured in the online database searches yielding 257 non-duplicate articles.

Studies were included if they met the following criteria: 1) used Cyberball behavioral paradigm as an experimental manipulation for social exclusion; 2) were empirical investigations (i.e. not review articles); 3) they employed fMRI; 4) reported group main effects of an exclusion/rejection condition relative to an inclusion/acceptance condition; 5) studied healthy subjects; and 6) used whole-brain analyses with reported Montreal Neurologic Institute (MNI) or Talairach coordinates. A flow chart illustration of the literature review and study selection process can be viewed in Fig. 2.1. Following the criteria defined above, 53 studies were included in the present study. It should be noted that 7 studies included in our final list involved participants watching others being excluded

(referred to hereafter as others-exclusion; ('I feel your pain: emotional closeness modulates neural responses to empathically experienced rejection', 2011; Lelieveld et al., 2020; Masten et al., 2011; Meyer et al., 2013; Novembre et al., 2015; Tousignant et al., 2018; van der Meulen et al., 2017), and two studies included in combined the whole-brain results for their clinical and healthy controls (Domsalla et al., 2014; van Harmelen et al., 2014). Analyses excluding these 9 studies are also provided.

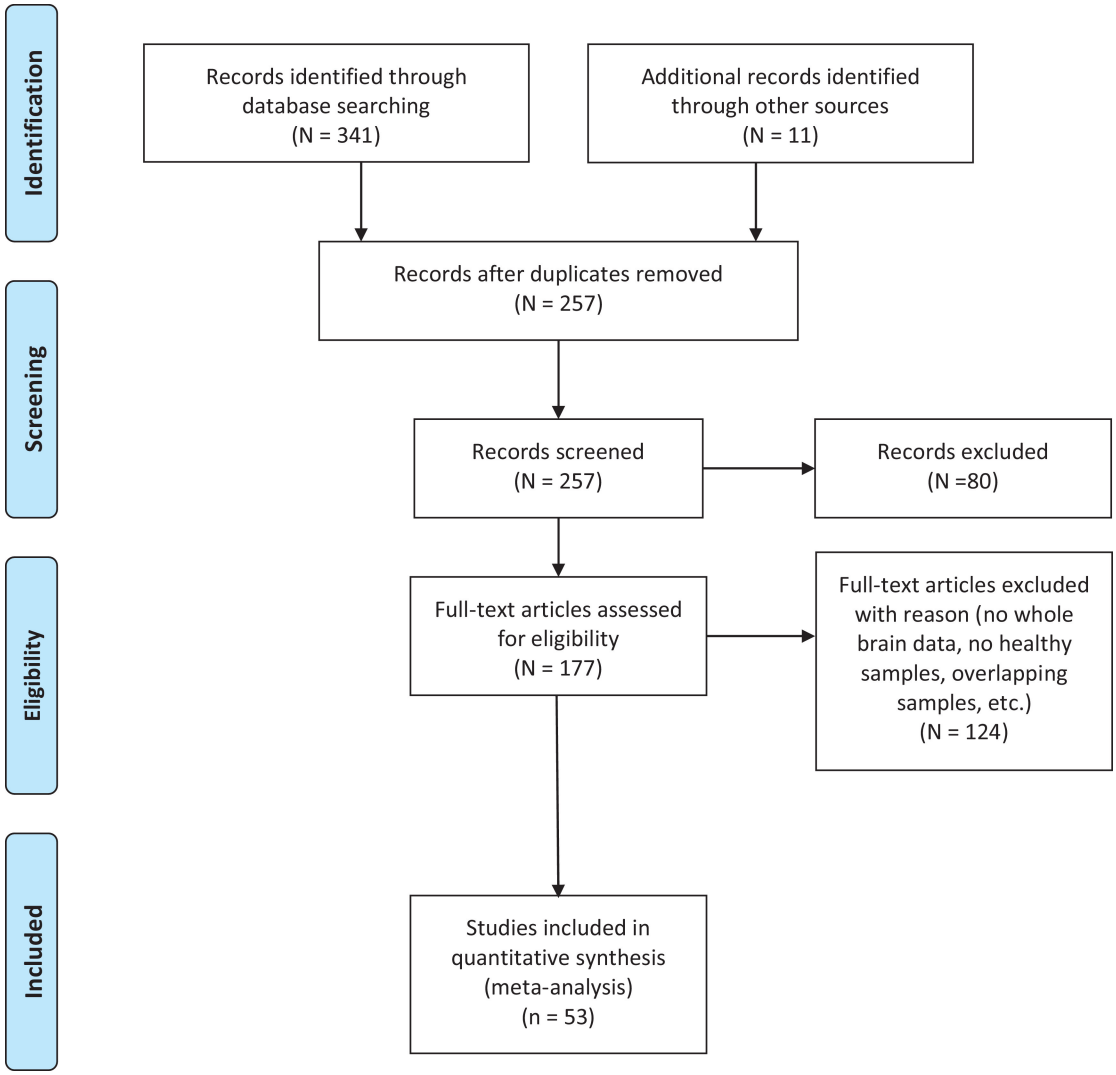

Figure 2.1: Caption. Flowchart of article selection, following PRISMA guidelines. Adapted from (Moher, 2009).

2.4.2 Coordinate based meta-analysis

Activation likelihood estimation (ALE) analysis

A coordinate-base meta-analysis of fMRI studies using Cyberball was conducted with the revised version of the ALE algorithm (Eickhoff et al., 2009, 2012). The software package GingerALE (3.0.2; www.brainmap.org/ale) was used to perform two analyses on coordinates from the studies identified by the literature search (Eickhoff et al., 2012; Laird et al., 2009). Coordinates from studies reporting in Talairach space were converted to MNI space using the FSL transformation applied in GingerALE (Eickhoff et al., 2012). ALE computes the statistical spatial convergence of activation coordinates (foci) across studies. The algorithm models this convergence by creating a 3-dimensional Gaussian distribution representing the spatial uncertainty around each coordinate. The width of the distribution is weighted by the number of participants for each study, such that studies with large sample sizes have smaller Gaussian distributions thereby reflecting a more reliable approximation of the true activation. Once a model of the brain activation map is computed for each study, the maps are aggregated to identify areas of spatial convergence between activation foci that are greater than would be expected by chance.

To better control for the false-positive rates, the ALE image was thresholded using two different thresholds. The first employed a conservative threshold ($p < 0.05$ FWE; 5000 permutations, $p < 0.001$ cluster forming threshold). A second, more liberal threshold used a cluster forming threshold of $p < 0.01$, a cluster-based family-wise error (FWE) corrected threshold of $p < 0.05$, and 5,000 permutations (Eickhoff et al., 2012). Significant clusters using the more conservative threshold were then used as seeds to perform a region-to-whole-brain co-activation meta-analysis (MACM; (Eickhoff et al., 2011; Laird et al., 2009).

Analysis

Five meta-analyses were performed using GingerALE: (1) full sample (53 studies, 1,817 participants); (2) traditional Cyberball design (29 studies, 1,021 participants); (3) adult

samples (33 studies, 1,094 participants); (4) alternating Cyberball design (17 studies; 565 participants); and (5) studies reporting statistically significant increased self-reported distress after exclusion (20 studies; 632 participants). Other than the meta-analysis on the full sample, all sub-analyse (2-5) did not include others-exclusion studies. For the full sample, we also provide results omitting 9 studies (7 studies of others-exclusion; 2 studies with combined whole brain results for healthy and clinical samples). The current recommendations for ALE meta-analyses is to include a minimum of 17–20 studies to obtain sufficient power to detect valid results from ALE analysis and to prevent results from being driven by a single experiment (Eickhoff et al., 2016; Müller et al., 2018). All meta-analyses satisfy this recommendation. To examine the effects of study design, we also performed a contrast analysis between the traditional and alternating Cyberball design. Although we were interested in examining the effects of age, due to the limited number of studies for the developmental sample (n = 13), a contrast analysis between age groups was not included due to insufficient power.

Meta-analytic connectivity modeling (MACM)

To provide a more comprehensive view of the co-activation pattern of brain regions associated with Cyberball task, we conducted MACM analyses for each ALE clusters. MACM allows for generating whole brain co-activation patterns for a given predefined region of interest across a range of experimental neuroimaging tasks and paradigms. Analogous to seed-based connectivity analysis of resting sate fMRI data, MACM assumes that regions that consistently coactivate across experiments can be pooled to create a map of functionally connected networks. Importantly, this approach is able to capture brain regions which are functionally connected, but that may also be part of an indirect network (Robinson et al., 2010). MACM leverages the BrianMap database (www.brainmap.org), a large online repository of human neuroimaging studies, to reveal brain regions that consistently activate together above chance with a given predefined region of interests across a large set of neuroimaging experiments (Eickhoff et al., 2011; Laird et al., 2009). We

created six different brain masks reflecting the six significant clusters obtained from the ALE meta-analysis cluster image from the full sample. Binarized brain masks for each cluster were generated using Nilearn (https://nilearn.github.io/index.html; (Abraham et al., 2014) on the basis of the voxel assignment corresponding to the ALE cluster they belong to. Sleuth (version 3.0.4, https://www.brainmap.org/sleuth) was used to search the BrainMap database for foci within each ALE cluster mask. Searches were conducted to include studies that reported increased activation. The search criteria were limited to statistical contrasts that reported activations (i.e. task $>$ baseline) in non-clinical populations. Studies that reported peak activation coordinates within each significant ALE cluster were assessed to establish each cluster's whole-brain co-activation pattern (cluster-level FWE$<$ 0.05; p-value$<$ 0.001; 5000 permutations).

2.4.3 Neurosynth cognitive decoder

After determining reliable activation patterns, we meta-analytically decoded the cognitive terms associated with this resulting ALE map from the full sample of 53 studies. Neurosynth is a meta-analytic tool that contains a database for over 14,000 functional neuroimage studies. The brain activation patterns and peak signal coordinates in the database are paired with associated cognitive terms (Yarkoni et al., 2011);
https://neurosynth.org). Taking a reverse inference approach, the Neurosynth decoder function was used to compare the activation pattern in our ALE map with those of all neuroimaging studies in the database. To do this, we first uploaded an unthresholded z-statistics map to NeuroVault which is a repository for neuroimaging studies. The Neurosynth decoder function is an integrated feature within NeuroVault, and was used it to compute a voxel-wise Pearson correlation coefficient between our ALE map each of the term-based z-statistics maps extracted from Neurosynth. The cognitive profile corresponding to the activation pattern from the meta-analysis was determined by identifying the most likely cognitive terms given activation in the ALE map. This produced a list of 1,335 terms, each with a correlation score to indicates the relative strength of association

with our ALE map. The top 20 terms (excluding all anatomical, redundant, and methodologic terms) were ranked by the correlation strength between the brain regions reliably engaged during social exclusion and Neurosynth maps, and visualized as a word cloud. The ALE map archived in NeuroVault (https://neurovault.org/collections/6199/) and can be used to generate the complete list of terms.

2.4.4 dACC study count

To compare the frequency with which published studies report dACC peak coordinates, we defined the boundaries of a dACC ROI using the Harvard Oxford probabilistic template (cingulate [anterior division] and paracingulate gyri posterior to the genu of the corpus callosum, $p > 0$). Foci were clustered into four categories based on the Harvard Oxford atlas: 1) studies reporting non-dACC peaks localized outside the dACC ROI, 2) studies reporting foci with the anatomical label dACC, but the coordinate fell outside of the ROI, 3) studies reporting dACC peaks that fell within the ROI, and 4) studies reporting foci that fell within the dACC ROI but were not given the dACC anatomical label.

2.5 Results

2.5.1 Meta-analysis on the full sample of Cyberball studies

Reliable patterns of brain activity were examined in 53 studies of Cyberball, revealing six clusters of activity (Table 2.2; Fig. 2.2). On the medial aspect of the frontal lobe, we found bilateral activation of vACC, extending anteriorly towards the ventral and medial prefrontal cortices. Cyberball exclusion reliably activated the right posterior insula, right superior frontal gyrus, left IFG, left posterior cingulate cortex (PCC), and left occipital pole. All ALE results images are archived in NeuroVault (https://neurovault.org/collections/6199/).

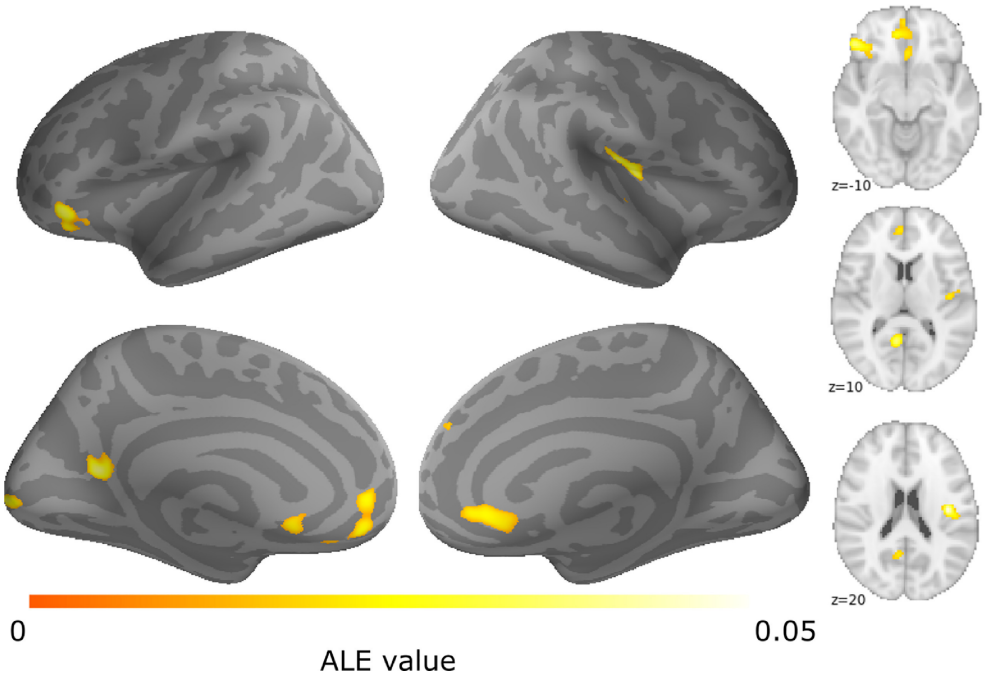

Figure 2.2: Caption. Results of cyberball social exclusion ALE meta-analysis. Brain areas showing consistent activation during social exclusion across (a) the full sample of Cyberball studies included in the meta-analysis (n=53).

Similar results were also observed when using a more liberal threshold (see Supplementary Figure 2.S1). When omitting the others-exclusion studies and the 2 studies that combined the whole-brain results of their healthy and clinical samples, all clusters except for the superior frontal gyrus remained (Supplementary Table 2.7).

2.5.2 Meta-analysis of Cyberball design

The next meta-analyses focused on Cyberball design to examine whether restricting the analysis to studies using the traditional Cyberball design (one round of inclusion followed by one round of exclusion) or the alternating design (repeated alternating blocks of inclusion and exclusion) to induce social exclusion might highlight a different activation pattern than that identified using the full Cyberball sample. Across all studies using the traditional design (n = 29; subjects = 1,021; foci = 300), ALE analysis identified a similar pattern of convergence as observed for the full sample. Social exclusion using the

traditional Cyberball design was associated with activity in three clusters identified in the full sample: left inferior frontal gyrus extending to the anterior insula, left occipital pole, and right superior frontal gyrus (see Supplementary Figure 2.S1, Supplementary Figure 2.S2 and Supplemental Table 2.2). The alternating design (n = 17, subjects = 565; foci = 170) was associated with the remaining two clusters identified in the full sample: the left vACC and right posterior insula. We did additionally find a cluster in the right central opercular cortex (see Supplementary Figure 2.S1, Supplementary Figure 2.S2 and Supplemental Table 2.2).

Contrast analyses between the traditional and alternating designs revealed reliably reported activity in left anterior insula for the traditional compared to the alternating design. The right central and parietal opercular cortex showed more reliable activation in the alternating relative to the traditional design (Supplementary Table 2.2).

2.5.3 Meta-analysis across studies of adults

To examine potential developmental effects in social exclusion, we conducted a meta-analysis of Cyberball studies using an adult population (n = 33; subjects = 1,094; foci = 350). The adult sample showed reliable activity in PCC, posterior insula, and subgenual and vACC (see Supplementary Figure 2.S1, Supplementary Figure 2.S3 and Supplementary Table 2.3). A preliminary analysis of children (Age less than 18 years old; n = 13; subjects = 480; foci = 121) is provided in supplemental material (Supplementary Figure 2.S3 and Supplementary Table 2.3).

2.5.4 Meta-analysis of self-reported distress

Further sub-analyses focused only on those studies where participants reported greater subjective experience of distress following exclusion (n = 20; subjects = 632; foci = 184) revealed similar clusters as for the full sample. Social exclusion in this sub-sample was

associated with engagement of the vACC, and bilateral IFG (see Supplementary Figure 2.S1, Supplementary Figure 2.S4 and Supplementary Table 2.S4).

2.5.5 Functional connectivity of the derived ALE-clusters—MACM analysis

To characterize the reliable activation associated with social exclusion, we examined the functional co-activation of the ALE map for the full sample with other brain regions. We performed MACM analyses to obtain cluster-specific connectivity maps that represent brain regions that coactivate with the largest and most reliable ALE cluster for the full sample (cluster1-bilateral vACC). The co-activation based meta-analytic map for cluster 1 is shown in Fig. 3 and corresponding peak maxima are reported in Table 3. ALE analysis examining the whole-brain co-activation pattern associated with the vACC showed co-activation with anterior and posterior cortical midline structures. Specifically, bilateral ventromedial prefrontal cortex extending towards the subgenual portion of the pregenual ACC, left superior frontal gyrus, and bilaterally in the PCC. The ACC cluster also coactivated with the left inferior parietal lobule, bilateral parahippocampal gyrus, and middle temporal gyrus. Furthermore, when depicted in conjunction with Yeo 7-Network atlas (Yeo et al., 2011), we show in Fig. 2.3 that the co-activation pattern for the ACC cluster aligns with the default network in medial prefrontal, as well as medial and lateral parietal cortex and temporal cortex. Supplementary Figures 2.S5-2.S9 and Supplementary Tables 2.S5-2.S9 show MACM results for clusters 5-6. MACM results images are archived in NeuroVault (https://neurovault.org/collections/6199/).

2.5.6 Cognitive terms associated activation

To provide valid reverse inference into the cognitive processes associated with the meta-analytic map for Cyberball, we performed functional decoding of the ALE results from the full sample. The Neurosynth decoder function was used assess the similarity of the

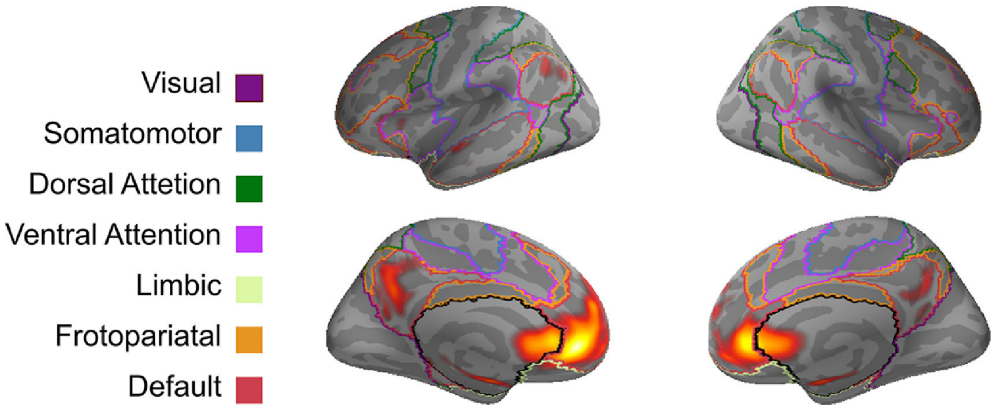

Figure 2.3: Caption. MACM map of co-activation of the vACC cluster (cluster 1) derived from the full sample ALE meta-analysis. Results represent the brain areas that significantly coactivate with brain regions that are most reliably recruited during social exclusion ($p < 0.001$, FWE cluster-level corrected at $p < 0.05$). The functional connectivity map for the vACC cluster is juxtaposed with outlines of the Yeo 7-Network atlas ((Yeo et al., 2011)). MACM co-activation pattern (yellow/red) overlap with the default network and portions the limbic network. See Table 3 for coordinates.

activation of the unthresholded ALE map with statistical maps generated for the entire set of terms included in the Neurosynth database. The top 20 Neurosynth cognitive terms with the highest correlation values are listed in Supplementary Table 10 and visualized as a word cloud in Fig. 2.4. The emerging pattern of ALE activation for the full sample was more associated with social- and self- cognition, as well as reward-related terms, such as: social, autobiographical, referential, mental states, reward, theory mind, value. The highest correlation was observed for the term social ($r = 0.18$).

2.5.7 dACC study count

Contrary to previous reports (Eisenberger, 2012a; Lieberman & Eisenberger, 2015), ALE results for the full sample and traditional sub-sample of Cyberball studies did not show reliable activation of the dACC. To further examine this inconsistency, we used a probabilistic atlas of the dACC to quantify the number of studies with reported peak voxels in the dACC. Less than one quarter of neuroimaging studies included in this meta-analysis

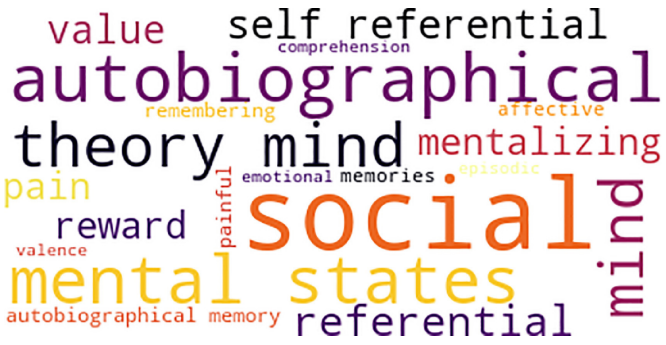

Figure 2.4: Caption. Decoding of the ALE map for the full sample using Neurosynth decoder. The decoder was used to compare the unthresholded ALE map (full sample) with statistical maps generated by Neurosynth across a wide range of terms (1,335 terms). Depicted above is the word cloud showing the top 20 relevant cognitive terms that correlated with the pattern of activation for social exclusion. Font size represents relative correlation strength of that term to the full sample Cyberball meta-analytic results.

reported anatomical labels for peak coordinates as the dACC (Fig. 2.5a). Of the 14 studies reporting dACC activity, the locations for nearly half of these studies (n = 6; Fig. 2.5a) did not have peak voxels located within the boundaries of the dACC (Fig. 2.5b). Four studies report coordinates within the dACC but provide a different anatomical label (e.g. medial prefrontal cortex).

2.6 Discussion

The present study conducted a coordinate based meta-analysis of social exclusion neuroimaging studies using the Cyberball paradigm. Using ALE, we found that social exclusion reliably engages bilateral vACC, right posterior insula, right superior frontal gyrus, left inferior frontal gyrus, left PCC, and left occipital pole. Similar patterns of activation were observed when restricting the analysis to studies to account for variable experimental manipulations and participant cohorts. Using a MACM approach to functionally characterize the pattern of co-activation from our ALE results, we demonstrate functional covariance of brain activity consistent with the topography of the default network. Im-

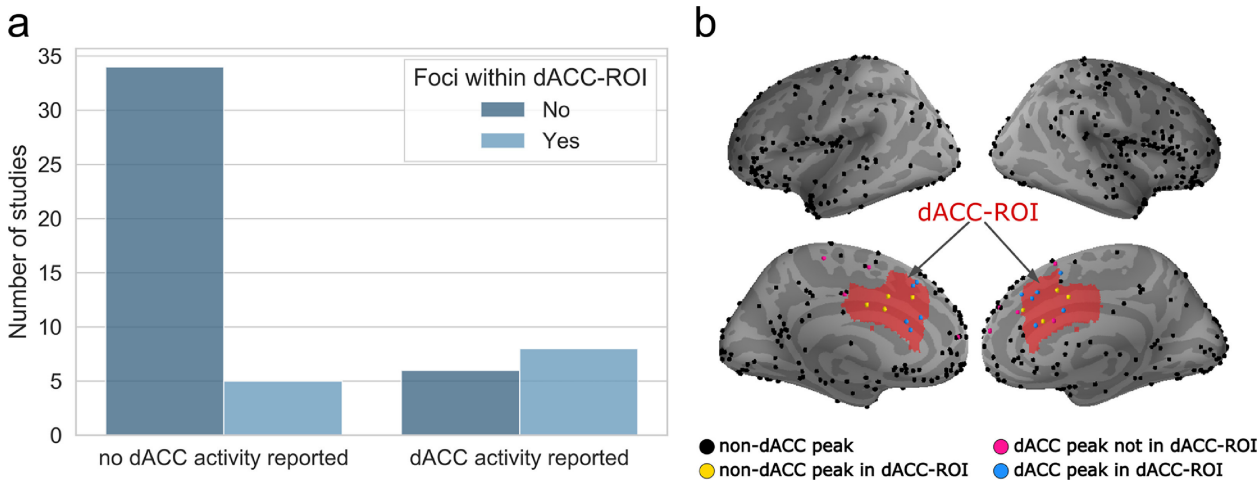

Figure 2.5: caption. Study count of reported dACC activity across all studies listed in Table 1.(a) A qualitative assessment comparing the number of studies that reported no peak activation in the dACC relative to studies that reported peak activation in the dACC during Cyberball social exclusion. Using a dACC-ROI map (created from the Harvard-Oxford probabilistic atlas) to cross-reference foci locations, studies with foci located outside of the dACC-ROI are portrayed in dark blue; those with foci located within the dACC-ROI in light blue. (b) Activation foci reported by studies included in the full sample ALE analysis are plotted on the brain surface. The red shaded area represents boundaries of a dACC ROI map. Non-dACC reported foci are color-coded base on whether they were located outside (black) or inside (yellow) of the ROI. Similarly, reported foci that were anatomical labeled as dACC located outside (pink) and inside (blue) the ROI are shown.

plementing valid reverse inference with Neurosynth, Cyberball activity was associated with social- and self-related cognitive terms, consistent with the functional role of the default network in cognition (Andrews-Hanna et al., 2014). While the neural response to social exclusion has been conceptualized within a social pain framework, strongly implicating the dACC, we found no converging evidence supporting dACC activation during social exclusion.

The meta-analysis results for the full sample indicate that several brain regions distributed broadly along the medial and lateral prefrontal cortices are consistently activated during Cyberball. In the prefrontal cortices, we found a large bilateral ventromedial pre-

frontal cluster including the pregenual and subgenual portions of the ACC (e.g. vACC). The ventral sub-region of the ACC has often been implicated in studies on emotion (Somerville et al., 2006). Increased activity in the vACC is associated with greater rejection sensitivity (Burklund et al., 2007), self-reported distress during social exclusion (Rotge et al., 2015), and engagement of this region may reflect emotional processing of negative emotions induced by social exclusion (Bolling, Pitskel, Deen, Crowley, Mayes et al., 2011; Sebastian et al., 2011). We also identified three additional clusters—one in the right posterior insula, a second in the right superior frontal gyrus, and a third in bilateral inferior frontal gyrus. The posterior insula is implicated in mediating sensorimotor processes of exteroceptive and interoceptive information (Chang et al., 2013; Craig, 2002; 'Placebo-induced changes in FMRI in the anticipation and experience of pain', 2004; Uddin, 2015), the inferior frontal gyrus plays a role in top-down cognitive control (Badre & Wagner, 2007), and the superior frontal gyrus which is encompassed within the dorsal medial prefrontal cortex commonly implicated in social-reflective tasks such as making judgements about others (Andrews-Hanna et al., 2014). The activation of these regions during social exclusion are linked to affective response and cognitive regulation of feelings of social exclusion (Bolling, Pitskel, Deen, Crowley, Mayes et al., 2011; Rotge et al., 2015; Sebastian et al., 2011). We also identified clusters in left PCC and left occipital pole. The PCC has strong anatomical connections with ventromedial prefrontal areas, and is a core hub of the default network (Andrews-Hanna et al., 2010). Together with the ventromedial areas, inferior and superior frontal gyri, these regions are part of the default network and are functionally integrated regions that support a wide range of self-generated cognitive processes, such as mentalizing and autobiographical recollection (Andrews-Hanna et al., 2014; Spreng et al., 2009). These default network regions are also responsive to visual social information during goal-oriented tasks (Spreng et al., 2014).

Neural correlates of social exclusion may be impacted by differences in methodological approaches, such as task design and participant populations of social rejection. Factors related to task design (Rotge et al., 2015; Somerville et al., 2006) and age-related

differences in rejection sensitivity (Somerville et al., 2006), and self-reported distress (Rotge et al., 2015) can differentially impact neural activity during exclusion. When restricting our analyses to Cyberball studies using the traditional and alternating designs, adult sample, and studies that reported significant increases in subjective distress after exclusion, we found similar clusters of activation as seen in the full sample.

Extending previous meta-analyses of neuroimaging studies using Cyberball, we conducting a MACM analysis with the observed bilateral vACC cluster as a seed region. The resulting meta-analytic functional connectivity map largely overlapped with regions of the default network (encompassing the medial prefrontal cortex, superior frontal gyrus, PCC, inferior parietal lobule, and hippocampus) and the orbitofrontal cortex, an extended region of the default network (Uddin et al., 2019). These results suggest that the functional co-activation pattern observed for this social exclusion cluster is spatially coherent with the default network.

Our results extend prior work by demonstrating that the functional characterization of regions reliably engaged during Cyberball coactivate with the default network which is known to be critical for reflective cognitive processes (Andrews-Hanna et al., 2014). They also add to a body of work linking social exclusion to a network of brain regions that are distinct from that previously identified in the extant literature on social pain. Using multivoxel pattern analysis, Woo et al. (2014) demonstrated that while the experience of social rejection and physical pain may engage similar brain areas, these experiences evoke dissociable functional connectivity patterns. When examining whole-brain network dynamics during Cyberball, social exclusion is associated with increased within-network connectivity of the default network ('Brain connectivity dynamics during social interaction reflect social network structure', 2017). The functional connectivity map derived for the vACC cluster results is largely consistent with this finding. Furthermore, our findings parallel results from seed-based connectivity showing increased connectivity between the vACC and default network brain regions during social exclusion (Bolling, Pitskel, Deen, Crowley, Mayes et al., 2011).

Using cognitive decoding to characterize the emerging pattern of ALE activation, we show that the cognitive processes primarily associated with the identified neural patterns relate to both social and self-referential cognitive terms, mentalizing and mental inference, and valence terms. The decoding results showed a small association between social exclusion task activity and 'pain' and 'painful'. Overall, these terms may capture spontaneous situational thoughts such as, "Why are they leaving me out?", which include mental state attribution and self-reference, along with the emotional experience of pain. Given that social exclusion is a complex phenomenon, this result underscores that the interplay between social cognitive and affective processing is an important component used to navigate a potentially challenging interpersonal situation. This may be particularly relevant in light of work pointing to increased emotion regulation processes following social exclusion (DeWall et al., 2011) and differential neural responsivity to positive and negative social feedback in regions implicated in social-affective processing (i.e. vACC) (Jankowski et al., 2018; Morese et al., 2019; Powers et al., 2013; Wagels et al., 2017). Collectively, our results highlight the importance of the default network in the experience of social exclusion by virtue of this network's involvement in self, social and emotional processes (Andrews-Hanna et al., 2014). Extending from this, a population neuroscience study has implicated the default network as central to the experience of loneliness (Spreng et al., 2020).

The neuroimaging literature on social exclusion has emphasized the role of the dACC in social pain, particularly given the association between activation in this region and subjective ratings of distress. The present ALE results show that social exclusion does not reliably engage the dACC, even when we lowered the statistical threshold for significance. One possibility for the lack of dACC engagement might be due to the task. Our results are limited to the Cyberball paradigm, yet other types of social exclusions paradigms have reported dACC activation (Gündel et al., 2003; O'Connor et al., 2008; Fisher et al., 2010; Kross et al., 2011; see Eisenberger et al., 2012b for review). Different paradigms might not evoke the same level of distress. Indeed, a prior meta-analysis on

social exclusion did find that the Cyberball task showing less dACC activity compared to other social exclusion tasks (Rotge et al., 2015). Taking this into consideration, when we restricted our analysis to studies where there was a significant increase in participants' self-reported distress, we still found no reliable dACC activity.

The dACC is often used by various studies as a region of interest, occasionally without providing any supplementary whole-brain analysis (Chester & DeWall, 2016; Chester et al., 2014; Dewall et al., 2010; Kashdan et al., 2014). It is possible that early observed effects (e.g. Eisenberger et al., 2003), with small samples by current standards, may have introduced a confirmation bias towards dACC, thereby obscuring findings of other brain regions that are more reliably recruited during social exclusion. Our findings underscore potential bias with the misattribution of observed peaks to the dACC, alongside the relatively sparse number of peaks within dACC.

The studies that report no dACC activity attribute the lack of replication to various factors such as differences in methodological approach (modified Cyberball, event-related design) or study population (adolescents versus adults) (Masten et al., 2011a; Masten et al., 2009; Moor et al., 2012; Sebastian et al., 2011). Others discuss their findings in terms of support for the ventral portion of the ACC's involvement in indexing negative affect and the dorsal portion being involved in cognitive control (Onoda et al., 2009; Shackman et al., 2011; Wagels et al., 2017). An alternate predominant view of dACC activation for social exclusion may have motivated bias in subsequent reports to fit their results in the social pain framework. Indeed, some studies without whole brain dACC effects conduct additional region of interest analysis on the dACC (Asscheman et al., 2019) or lowered the statistical threshold for significance (Bollings et al., 2011b). Analyses investigating neural correlations with self-reported distress can more directly speak to the participant's neural response to the experience of social exclusion. However, correlations between distress and the dACC have been inconsistent (DeWall et al., 2012; Eisenberger, 2012a; Kawamoto et al., 2012; Masten et al., 2011; Masten et al., 2009; Moor et al., 2012; Onoda et al., 2010; Will et al., 2015). As the field moves forward in characterizing the

neural correlates of social exclusion, it is critically important that reliable observations of brain activity be considered above confirmation bias.

The main advantage of taking a coordinate-based meta-analytic approach is that it is data-driven and is considered a robust method for unbiased integration of previously published functional neuroimaging literature. However, some important limitations should be acknowledged. First, we pooled across neuroimaging studies using the Cyberball task (both traditional and alternating design). We did not include studies utilizing other social exclusion paradigms. While our findings are largely consistent with previous meta-analyses which did include analyses using other social exclusion paradigms (Cacioppo et al., 2013; Vijayakumar et al., 2017), inferences regarding the meaning of the findings may not be generalizable to other social exclusion paradigms (i.e. romantic rejection, viewing visual stimuli of rejection). In pooling peak coordinates reported in published activation tables, the shape and extent of the primary result clusters in a volume may not be well-characterized. This will likely result in imperfect alignment of activity across studies. However, in the absence of consistently archived results images, coordinate based meta-analysis remains the most effective approach to amalgamating neuroimaging finds across studies. Another important limitation is that while we did provide a sub-analysis focused on studies where there was statistical evidence of greater subjective distress following exclusion, this analysis does not directly speak to how social distress correlates with brain activity. We recommend that future studies perform whole-brain regression analyses with self-reported distress to more directly identify which brain regions are involved in the affective response to social exclusion. Finally, the Neurosynth decoder is constrained by the term-based maps in the database (Yarkoni et al., 2011). Neurosynth automatically extract high frequency terms from the abstract of each study in the database, which can impact the specificity of cognitive terms. While the terms from the cognitive decoding complement our interpretation of an association between social exclusion and default network recruitment, we do not claim that there is a unique role for activity in any of the brain regions identified in this meta-analysis and cognitive terms obtained from our analysis.

The correlation values represent how well the spatial distribution of activation associated with each term in the database matches the reliable activation patterns of our ALE result map. Despite these limitations, Neurosynth represents a powerful tool for decoding cognitive terms and has been shown to have high sensitivity and specificity for identify distinct neural networks (see Rubin et al., 2017; Yarkoni et al.,2011). The functional characterization results are useful for developing hypotheses that provide a better fit to the data, and allow the field to move forward towards a better understanding of the neural and cognitive-affective basis of social exclusion.

In summary, the current meta-analysis of Cyberball reveals a reliable pattern of brain activation distributed across medial prefrontal cortex, inferior and superior frontal gyri, posterior cingulate cortex and posterior insula. This pattern largely overlaps with the default network, and is associated with self-referential processes, mentalizing and valence related terms. Together, these results provide evidence for a primary role of the default network in the experience of social exclusion.

2.7 Data and code availability statement

All data are accessible within the studies cited in Table 2.7. Extracted coordinates are available from either author upon request.

Table 2.1: Cyberball studies meeting inclusion criteria.

No.	Reference	N	Gender ratio (F/M)	Age M(sd)	Cyberball design	Age group	Target of exclusion	Increased self-reported distress after exclusion	N (foci)	dACC activity reported	Peaks in dACC ROI
1	Ascheman et al., 2019	55.0	(0/55)	10.40(0.74)	Traditional	Developmental	Self	yes	6.0	no	no
2	Bach et al., 2019	21.0	(2/19)	1:38.53(6.5) **2:38.19(8.1**)	Alternating	Adult	Self	yes	9.0	no	no
3	Beeney et al., 2011	20.0	(20/0)	24.6(5.8)	Traditional	Adult	Others		9.0	yes	yes
4	Bolling et al., 2011a	21.0	(6/15)	12.90(2.59)	Alternating	Developmental	Self	yes[b]	10.0	no	no
5	Bolling et al., 2011b	23.0	(12/11)	24.0(3.81)	Alternating (picture)	Adult	Self	no	12.0	no	no
6	Bolling et al., 2012	20.0	(9/11)	24.99 (3.91)	Alternating (picture)	Adult	Self	yes[b]	12.0	no	no
7	Bolling et al., 2015	15.0	(6/9)	1:12.96(2.7) 2:12.36(4.2) **3:11.88 (3.2)**	Alternating	Developmental	Self	yes[b]	12.0	no	yes
8	Bolling et al., 2016	20.0	(10/10)	12.61(2.5)	Alternating	Developmental	Self	yes[b]	12.0	no	no
9	Bonenberger et al., 2015	21.0	(21/0)	22.2(3.38)	Traditional	Adult	Self	no	8.0	no	no
10	Cheng et al., 2020	69.0	(36/33)	14.2(1.5)	Alternating	Developmental	Self	yes[b]	4.0	no	yes
11	Chester et al., 2018	60.0	(38/22)	20.28(2.77)	Traditional	Adult	Self	no	19.0	no	no
12	Cogoni et al., 2018	36.0	(19/17)	23.2(3.51)	Alternating (video)	Adult	Self	no	14.0	no	no
13	Domsalla et al., 2014	20.0	(20/ 0)	1:29(2) 2:28.7(7.8)	Alternating	Adult	Self	yes[b]	12.0	no	no
14	de Water et al., 2017	31.0	(17/14)	14.49(1.06)	Alternating	Developmental	Self	yes	7.0	no	yes
15	DeWall et al., 2012	25.0	(16/9)	Undergraduate	Traditional	Adult	Self	yes[b]	15.0	no	no

16	Eisenberger et al., 2003	13.0	(9/4)	Undergraduate	Traditional	Adult	Self	yes	4.0	yes	yes
17	Falk et al., 2014	36.0	(36/0)	16.8(0.47)	Traditional	Developmental	Self	yes	6.0	no	no
18	Gilman et al., 2016	42.0	(22/20)	1:21.5(1.9) 2:20.6(2.5)	Traditional	Adult	Self	yes[b]	5.0	no	no
19	Gonzalez et al., 2015	85.0	(45/40)	24.5(1.35)	Traditional	Adult	Self	no	6.0	yes	yes
20	Gradin et al., 2012	16.0	(9/7)	**1:40.87(11.72)** 2:41.23(11.78)	Alternating	Adult	Self	yes[b]	3.0	no	no
21	Hanlon et al., 2019	25.0	(14/11)	1:33.5(6) 2:38.1(6.1)	Traditional	Adult	Self	yes	1.0	yes	yes
22	Heeren et al., 2017	23.0	(23/0)	1:24.96(6/6) **2:25.30(5.62)**	Traditional (events)	Adult	Self	yes	5.0	yes	yes
23	Karramans et al., 2011	15.0	(20/5)	22 (19–33)	Traditional	Adult	Self	yes[b]	13.0	no	no
24	Kawamoto et al., 2012	22.0	(19/3)	20.7(1.7)	Alternating (events)	Adult	Self	yes	12.0	yes	yes
25	Le et al., 2020	64.0	(33/31)	47.1(16.3)	Traditional	Adult	Self	no	27.0	yes	yes
26	Lelieveld et al., 2012	30.0	(16/14)	20.00(1.05)	Traditional	Adult	Self	no	3.0	no	no
27	Lelieveld et al., 2020	43.0	(25/18)	20.95(1.89)	Traditional	Adult	Others		3.0	yes	no
28	Luo et al., 2016	42.0	(21/21)	1:20.38(1.7) 2:20.38(1.12)	Traditional	Adult	Self	yes	16.0	yes	yes
29	Malejko et al., 2018	17.0	(17/0)	**1:23(4.26)** 2:23.3(4.13) 3:28.7(4.59)	Traditional	Adult	Self	yes[b]	11.0	no	no
30	Masten et al., 2010	23.0	(14/9)	13.0(no sd)	Traditional	Developmental	Self	no	4.0	no	no
31	Masten et al., 2011a	18.0	(9/9)	21.4(no sd)	Traditional	Adult	Self	yes[b]	15.0	no	no
32	Masten et al., 2011b	18.0	(9/9)	20.22(no sd)	Traditional	Adult	Others		5.0	no	no
33	Masten et al., 2011c	17.0	(2/15)	1:14.0(2.4) 2:13.6(2.5)	Traditional	Developmental	Self	yes	19.0	no	no
34	Maurage et al., 2012	22.0	(0/22)	1:45.1(10.69) 2: 47.2(11.04)	Traditional	Adult	Self	yes	7.0	yes	yes

35	McIver et al., 2018	45.0	(36/9)	17.7(0.60)	Traditional	Developmental	Self	yes[b]	11.0	yes	yes
36	Meyer et al., 2013	16.0	(12/4)	21.69(2.12)	Traditional (picture)	Adult	Others		7.0	yes	yes
37	Moor et al., 2010	53.0	(31/22)	1:11.8(.87) 2:15.74(0.74) 3:20.38(0.85)	Traditional (events)	Developmental	Self	yes	9.0	no	no
38	Nishiyama et al., 2015	46.0	(29/17)	19.85(no sd)	Traditional	Adult	Self	yes	14.0	no	no
39	Novembre et al., 2015	23.0	(23/0)	22.4(2.0)	Alternating	Adult	Self & Others		13.0	no	yes
40	Olié et al., 2017	28.0	(28/0)	**1:38.9(no sd)** 2:41(no sd) 3:36(no sd)	Traditional	Adult	Self	yes[b]	1.0	no	no
41	Onoda et al., 2009	26.0	(15/11)	21.7(1.3)	Traditional	Adult	Self	yes	2.0	no	no
42	Preller et al., 2016	21.0	(9/12)	26.48(4.76)	Traditional	Adult	Self	no	26.0	yes	yes
43	Puetz et al., 2016	51.0	(26/25)	1:10.6(1.75) 2:10.38(1.67)	Traditional	Developmental	Self	yes[b]	2.0	no	no
44	Radke et al., 2018	80.0	(40/40)	1:24.38(3.37) 2:24.69(3.85)	Alternating	Adult	Self	no	5.0	no	no
45	Sebastian et al., 2011	35.0	(35/0)	1:15.44(0.81) 2:28.70(3.91)	Alternating	Developmental & Adult	Self	yes	12.0	no	no
46	Tousignant et al., 2018	40.0	(20/20)	1:14.25(1.33) 2:24.25(1.97)	Alternating (picture)	Developmental & Adult	Others		9.0	yes	yes
47	van den Berg et al., 2018	72.0	(44/28)	36.2(16.17)	Alternating	Adult	Self	no	11.0	no	yes
48	van der Meulen et al., 2017	71.0	(39/32)	1:8.15(1.06) 2:8.23(0.67) 3:8.21(0.71)	Traditional	Developmental	Others		29.0	no	no
49	van Harmelen et al., 2014	46.0	(34/12)	1:18.31(1.95) 2:18.85(0.25)	Traditional	Adult	Self	yes	8.0	no	no
50	Wagels et al., 2017	40.0	(20/20)	27.80(7.86)	Alternating	Adult	Self	no	20.0	no	no

51	Will et al., 2015	28.0	(16/12)	20.7(1.97)	Traditional (event)	Adult	Self	yes	13.0	no	no
52	Will et al., 2016	44.0	(18/26)	14.0(0.70)	Traditional (event)	Developmental	Self	yes	19.0	no	no
53	Wudarczyk et al., 2015	24.0	(10/14)	24.33(2.91)	Alternating	Adult	Self	yes	3.0	no	no

Note: For Age M(sd), bolded items represent group(s) whose data was included in the meta-analysis. “Alternating” involves Cyberball design with alternating inclusion and exclusion blocks. Modifications made in the Cyberball design are indicated in parentheses (e.g. using an event-related design, providing a picture of a person to represent the other Cyberball players, etc.). “Self” involves participants being excluded while “Others” involves participants watching friends or strangers being excluded.

[a] There is a discrepancy between the total number of subjects listed and the gender ratio listed in this reference.

[b] Studies that provided average self-reported distress scores but did not perform any statistically assessment of social distress after exclusion.

Table 2.2: Results of the full sample ALE meta-analysis.

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)	N Studies (foci)
vACC	L	-2.0	42.0	-14.0	0.03	1	5384.0	21(34)
Posterior insula	R	40.0	-14.0	18.0	0.06	2	2296.0	11(15)
IFG	L	-48.0	34.0	-10.0	0.04	3	2264.0	11(13)
PCC/RSC	L	-8.0	-56.0	12.0	0.04	4	1523.0	8(8)
Occipital pole	L	-12.0	-94.0	4.0	0.04	5	1112.0	6(8)
SFG	R	2.0	50.0	30.0	0.02	6	848.0	5(8)

Note: Results of the full sample ALE meta-analysis. For each cluster, brain region label, hemisphere, MNI coordinates, ALE maxima , cluster size (mm3), and number of studies are provided. vACC = ventral anterior cingulate cortex; IFG= inferior frontal gyrus; PCC = posterior cingulate cortex; RSC = retrosplenial cortex; SFG= superior frontal gyrus; L = left hemisphere; R= right hemisphere.

Table 2.3: Cluster 1 MACM result: clusters of functional co-activation associated with ALE cluster 1 (vACC-medial prefrontal cortex) from the full sample ALE meta-analysis. Brain region label, hemisphere, MNI coordinates, ALE value, and cluster size (mm3)

Brain Region	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)
vACC	L	-2	46	-12	0.30	1	48264
dorsal PCC	L	-2	-52	29	0.10	2	14312
Parahippocampal gyrus	L	-20	-8	-20	0.10	3	5104
Inferior parietal lobule	L	-42	-76	34	0.08	4	5080
Parahippocampal gyrus	R	22	-6	-20	0.10	5	4792
Orbitofrontal cortex	L	-40	24	-14	0.06	6	3408
Superior frontal gyrus	L	-22	32	44	0.07	7	3408
Middle temporal gyrus	L	-56	-10	-18	0.07	8	1488

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space

All data were analyzed with software publicly available from http://www.brainmap.org/ and https://neurosynth.org/.

2.8 Credit author statement

Mwilambwe-Tshilobo: Conceptualization, Methodology, Investigation, Data Curation, Formal analysis, Visualization, Writing

Spreng: Conceptualization, Methodology, Writing, Supervision

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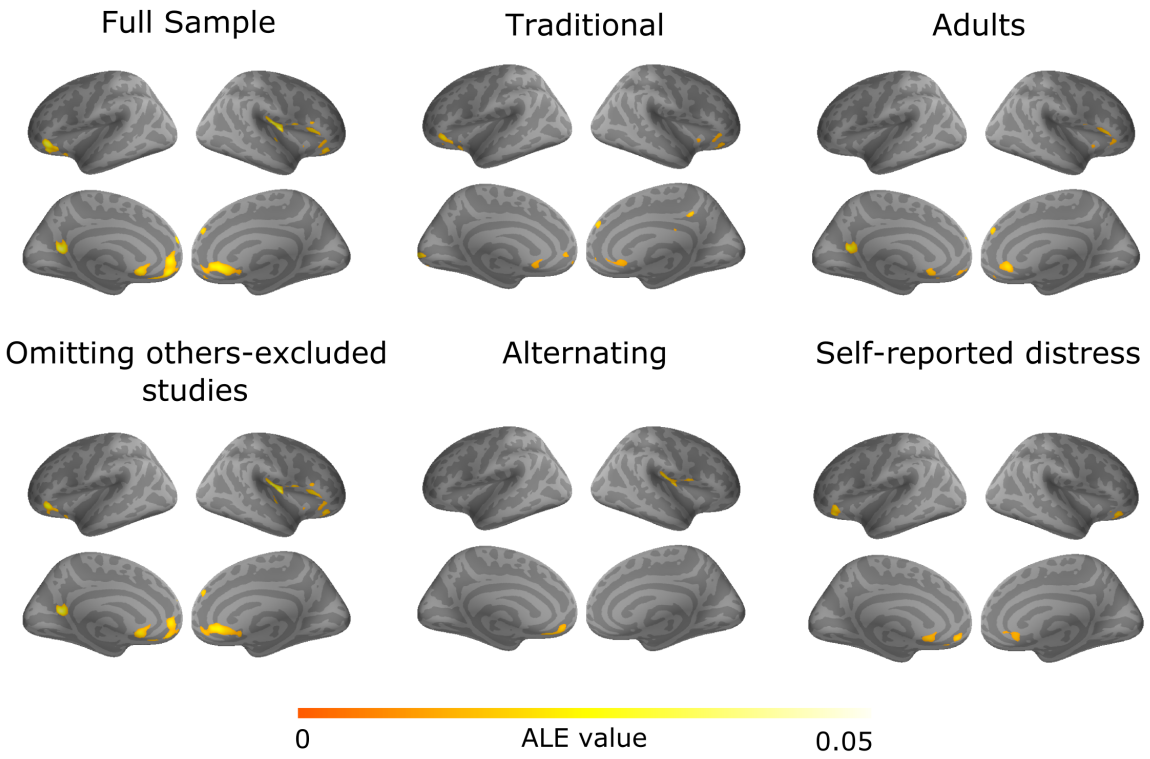
2.11 Appendix. Supplementary materials



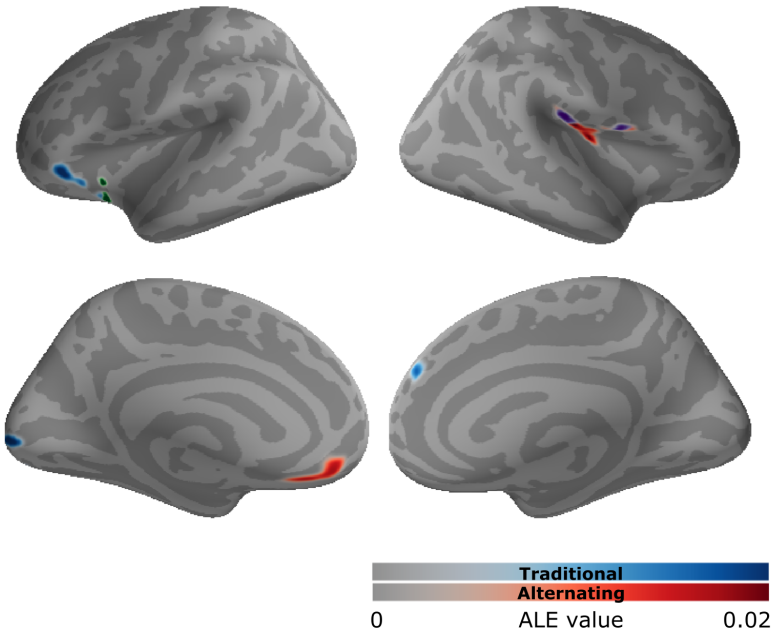
Supplementary Figure 2.S1: Full sample, traditional design, adult sample ALE sub-analyses. Meta-analytic results for the full sample, traditional design, adult sample, Cyberball studies omitting all studies where participants observing other's being excluded, alternating design, and studies that reported statistically significant levels of distress after exclusion (self-reported distress). All ALE analyses were thresholded at $p < .05$ using a FWE correction at the cluster level, corrected for multiple comparisons (5,000 permutations) with a cluster forming threshold of $p < .01$.

Table 2.S1: ALE analysis omitting others-exclusion studies and studies of combined whole-brain results for healthy and clinical samples.

ALE analysis omitting others-exclusion studies and studies of combined whole-brain results for healthy and clinical samples (n=44; 1,508 participants; 451 foci).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)	N Studies (foci)
vACC	R	6.0	38.0	-6.0	0.03	1.0	3440.0	15(21)
Posterior insula	R	40.0	-14.0	18.0	0.05	2.0	2208.0	10(13)
IFG	L	-48.0	34.0	-10.0	0.03	3.0	1424.0	8(10)
PCC/RSC	L	-8.0	-56.0	12.0	0.03	4.0	1048.0	6(6)
Occipital pole	L	-12.0	-94.0	4.0	0.03	5.0	880.0	5(6)

*Note.*For each cluster, brain region label, hemisphere, MNI coordinates, associated ALE maxima, cluster size (mm3), and number of studies are provided. L = left hemisphere; R= right hemisphere. x, y, z coordinates provided in MNI space.



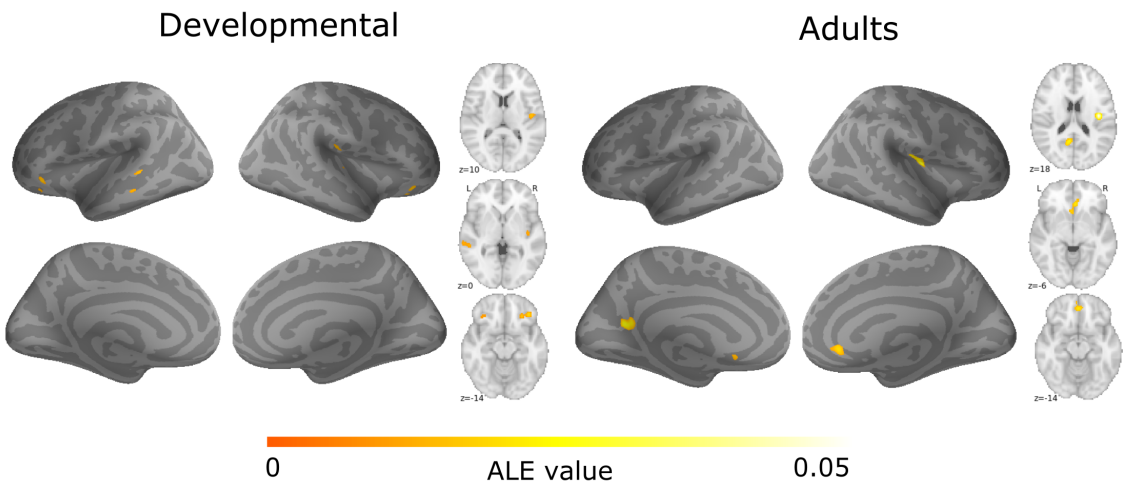
Supplementary Figure 2.S2: ALE meta-analysis results for the design Cyberball sub-analysis. Brain areas showing consistent activation during social exclusion for studies using the traditional design (1 round of inclusion followed by 1 round of exclusion) and alternating design (alternating rounds of inclusion and exclusion) are depicted. Regions in green represent areas of greater activation for the traditional relative to the alternating. Brain regions showing greater activation during the alternating compared to the traditional are depicted in purple.

Table 2.S2: Results from the ALE analyses of Cyberball design.

Results from the ALE analyses of Cyberball design.

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)	N Studies (foci)
Traditional Cyberball								
Inferior frontal gyrus	L	-36.0	24.0	-6.0	0.03	1.0	1720.0	7(8)
Superior frontal gyrus	R	4.0	46.0	32.0	0.02	2.0	1144.0	5(9)
Occipital pole	L	-12.0	-94.0	-6.0	0.03	3.0	888.0	5(6)
Alternating Cyberball								
Ventral anterior cingulate cortex	L	-4.0	42.0	-14.0	0.02	1.0	1952.0	7(9)
Posterior insula	R	40.0	-16.0	18.0	0.03	2.0	1888.0	7(7)
Central opercular/posterior insula	R	56.0	4.0	4.0	0.02	3.0	1192.0	6(7)
Traditional $>$ *Alternating*								
Anterior insula	L	-36.0	20.0	-4.0	2.72	1.0	232.0	2(2)
Alternating $>$ *Traditional*								
Central opercular cortex	R	56.0	2.0	8.0	3.09	1.0	696.0	3(3)
Parietal opercular cortex	R	48.0	-22.0	16.0	3.54	2.0	648.0	2(2)

For each cluster, brain region label, hemisphere, MNI coordinates, associated ALE maxima, cluster size (mm3), and number of studies are provided. L = left hemisphere; R= right hemisphere.



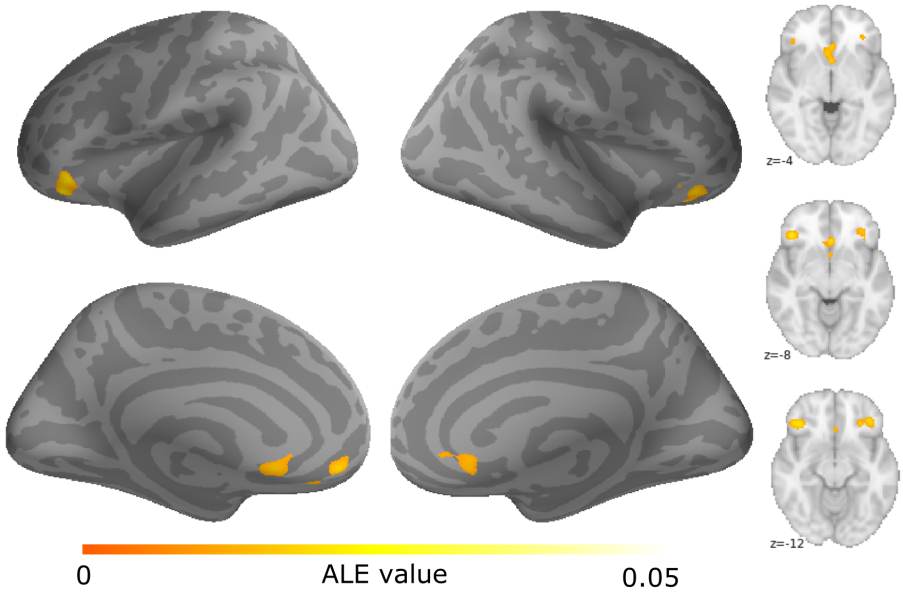
Supplementary Figure 2.S3: ALE meta-analysis results for Cyberball studies where participants reported significantly greater levels of distress after exclusion. ALE meta-analysis results for Cyberball studies where participants reported significantly greater levels of distress after exclusion.

Table 2.S3: ALE analysis on Cyberball studies in developmental and adult samples.

ALE analysis on Cyberball studies in developmental and adult samples. Brain region label, hemisphere, MNI coordinates, ALE maxima, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)	N Studies (foci)
Developmental								
Middle temporal gyrus	L	-58.0	-30.0	-6.0	0.02	1.0	1232.0	6(6)
Inferior frontal gyrus	L	-44.0	32.0	-8.0	0.02	2.0	1080.0	4(6)
Inferior frontal gyrus	R	38.0	34.0	-14.0	0.02	3.0	1024.0	4(5)
Posterior insula	R	44.0	-14.0	8.0	0.02	4.0	928.0	3(5)
Parietal opercular cortex	R	46.0	-20.0	20.0	0.02	5.0	608.0	3(3)
Adults								
Posterior cingulate cortex	L	-8.0	-56.0	14.0	0.03	1.0	1312.0	7(7)
Posterior insula	R	40.0	-14.0	18.0	0.05	2.0	1168.0	6(6)
Subgenual anterior cingulate-	R	4.0	32.0	-6.0	0.02	3.0	1024.0	6(8)
Ventral anterior cingulate	R	0.0	44.0	-14.0	0.02	4.0	888.0	5(5)

Note. For each cluster, brain region label, hemisphere, MNI coordinates, associated ALE maxima, cluster size (mm3), and number of studies are provided. L = left hemisphere; R= right hemisphere. x, y, z coordinates provided in MNI space.



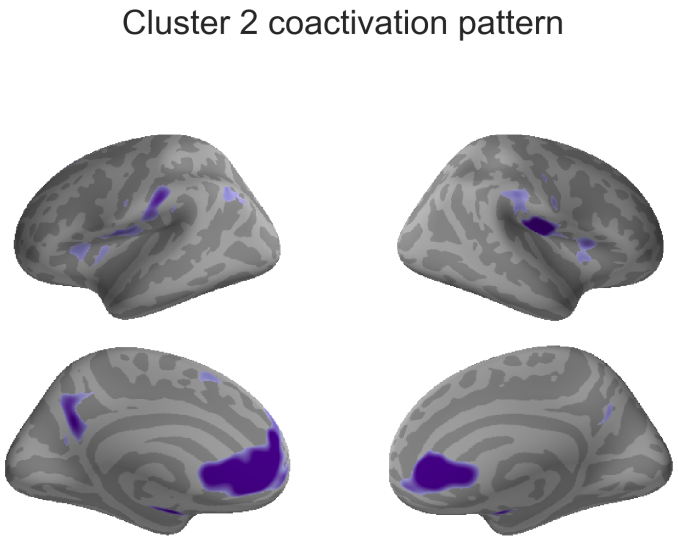
Supplementary Figure 2.S4: ALE meta-analysis results for the adult sub-analysis. Brain areas showing consistent activation during social exclusion in developmental (ages < 18y) and adult samples (ages 18y+).

Table 2.S4: Distress ALE sub-analysis

ALE analysis on Cyberball studies that found greater self-reported distress levels after exclusion. Brain region label, hemisphere, MNI coordinates, ALE maxima, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)	N Studies (foci)
Ventral anterior cingulate	R	2	26	-8	0.02	1	1544	7(7)
Inferior frontal gyrus	L	-42	32	-10	0.02	2	1424	5(7)
Inferior frontal gyrus	R	38	34	-14	0.02	3	1400	5(6)

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space.



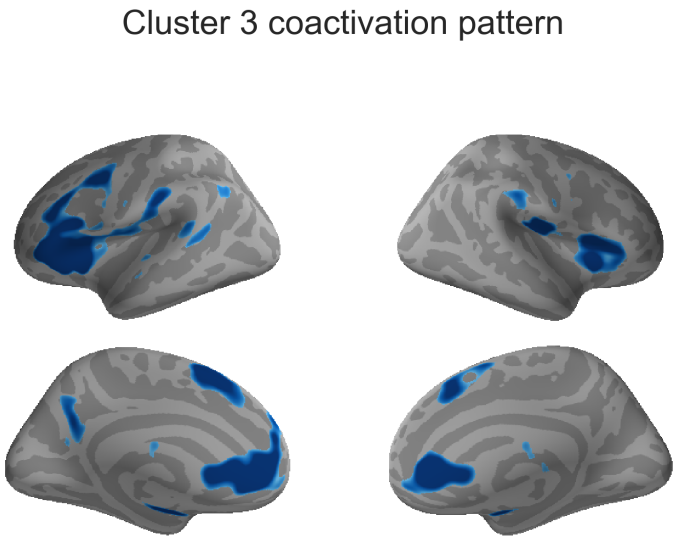
Supplementary Figure 2.S5: MACM map of coactivation of the right posterior insula cluster (cluster 2) derived from full sample ALE meta-analysis displayed on the brain surface.

Table 2.S5: MACM cluster 2 results

Cluster 2 MACM results: clusters of functional coactivation associated with right anterior insula cluster (cluster 2) from the full sample ALE meta-analysis. Brain region label, hemisphere, MNI coordinates, ALE value, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)
Ventromedial PFC	L	-2	46	-12	0.31	1	42088
Posterior insula	R	40	-16	16	0.23	2	20024
Parietal operculum	L	-56	-24	20	0.10	3	18808
Posterior cingulate cortex	L	-8	-56	18	0.11	4	10888
Dorsal anterior cingulate	L	0	8	44	0.09	5	7208
Lateral occipital cortex	L	-42	-76	32	0.08	6	4808
Amygdala	L	-20	-8	-20	0.12	7	3456
Amygdala	R	22	-6	-20	0.10	8	3216
Thalamus	R	14	-14	8	0.11	9	2840
Thalamus	L	-12	-16	6	0.09	10	2088
Superior frontal gyrus	L	-22	32	44	0.08	11	1960

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space.



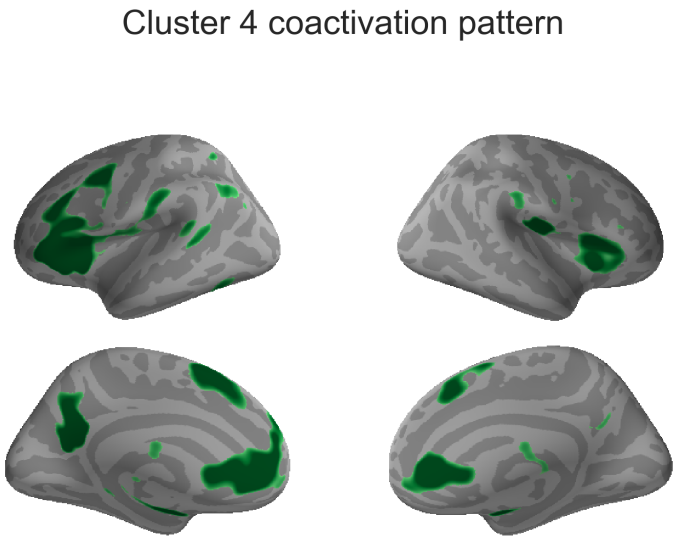
Supplementary Figure 2.S6: MACM map of coactivation of the left inferior frontal gyrus cluster (cluster 3) derived from full sample ALE meta-analysis displayed on the brain surface.

Table 2.S6: MACM cluster 3 results

Results of the MACM: clusters of functional coactivation associated with left inferior frontal gyrus cluster (cluster 3) from the full sample ALE meta-analysis. Brain region label, hemisphere, MNI coordinates, ALE value, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)
Inferior frontal gyrus	L	-36	24	-6	0.47	1	52656
Ventral anterior cingulate cortex	L	-2	46	-12	0.26	2	48600
Paracingulate gyrus	L	-4	18	44	0.17	3	18904
Inferior frontal gyrus	R	38	24	-8	0.21	4	13368
Posterior insula	R	40	-16	16	0.22	5	11608
Posterior cingulate/retrosplenial cortex	L	-6	-56	18	0.11	6	6008
Lateral occipital cortex	L	-42	-76	32	0.08	7	3176
Amygdala	R	22	-6	-18	0.13	8	2680
Middle frontal gyrus	R	52	10	36	0.08	9	2104

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space.



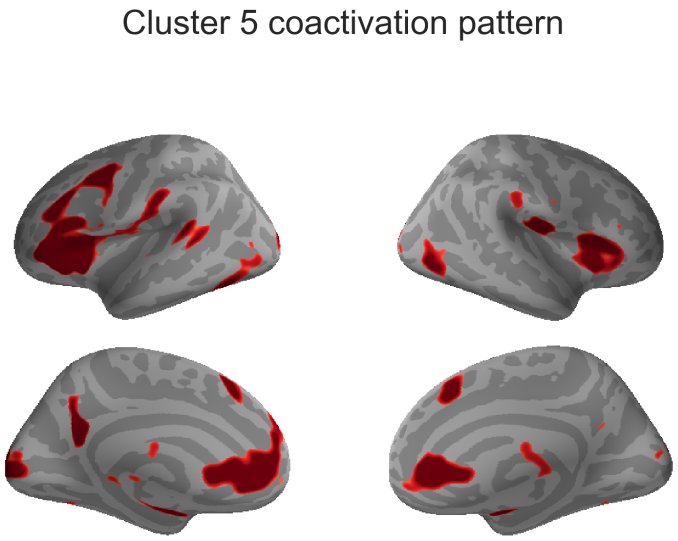
Supplementary Figure 2.S7: MACM map of coactivation of the left posterior cingulate cortex cluster (cluster 4) derived from full sample ALE meta-analysis displayed on the brain surface.

Table 2.S7: MACM cluster 4 results

Results of the MACM: clusters of functional coactivation associated with left posterior cingulate cortex cluster (cluster 4) from the full sample ALE meta-analysis. Brain region label, hemisphere, MNI coordinates, ALE value, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)
Ventral anterior cingulate cortex	L	-2	48	-12	0.25	1	50464
Inferior frontal gyrus	L	-36	24	-6	0.46	2	45768
Paracingulate gyrus	L	-4	14	50	0.17	3	18496
Inferior frontal gyrus	R	38	24	-8	0.17	4	12288
Posterior cingulate cortex/retrosplenial cortex	L	-8	-56	14	0.26	5	10800
Posterior insula	R	40	-16	16	0.22	6	10456
Lateral occipital cortex	L	-42	-76	34	0.10	7	4992
Superior temporal gyrus	L	-54	-42	6	0.11	8	3208
Amygdala	R	22	-6	-20	0.14	9	2912
Precentral gyrus	R	50	10	34	0.09	10	2552
Temporal occipital fusiform cortex	L	-40	-48	-22	0.10	11	2120
Superior lateral occipital cortex	L	-28	-64	50	0.10	12	1880

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space.



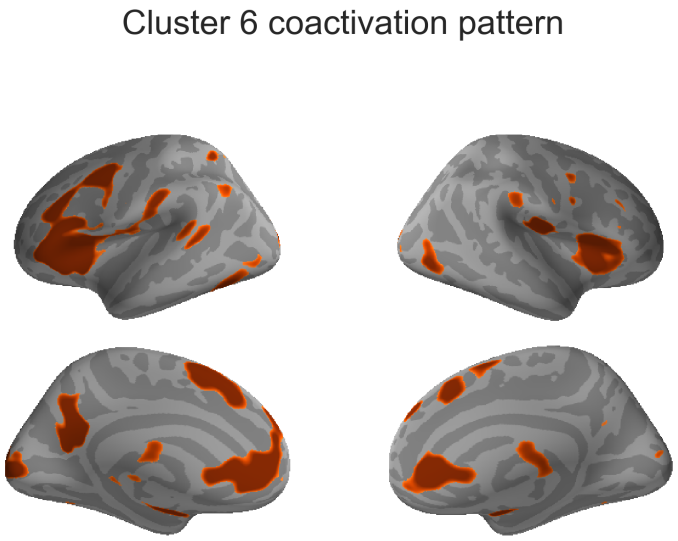
Supplementary Figure 2.S8: MACM map of coactivation of the left occipital pole (cluster 5) derived from full sample ALE meta-analysis displayed on the brain surface.

Table 2.S8: MACM cluster 5 results

Results of the MACM: clusters of functional coactivation associated with the left occipital pole (cluster 5) from the full sample ALE meta-analysis. Brain region label, hemisphere, MNI coordinates, ALE value, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)
Inferior frontal gyrus	L	-36	24	-6	0.45	1	97192
Occipital pole	L	-12	-92	0	0.28	2	13632
Inferior frontal gyrus	R	38	24	-8	0.23	3	12104
Posterior insula	R	40	-16	16	0.21	4	9600
Temporal occipital fusiform cortex	L	-38	-56	-16	0.12	5	6080
Paracingulate gyrus	L	-4	24	44	0.15	6	4808
Inferior lateral occipital cortex	R	42	-72	-10	0.12	7	4504
Posterior cingulate/retrosplenial cortex	L	-8	-56	14	0.25	8	4408
Paracingulate gyrus	L	-2	18	42	0.18	9	2656
Amygdala	L	22	-6	-20	0.15	10	2312

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space.



Supplementary Figure 2.S9: MACM map of coactivation of the right superior frontal gyrus (cluster 6) derived from full sample ALE meta-analysis displayed on the brain surface.

Table 2.S9: MACM cluster 6 results

Results of the MACM: clusters of functional coactivation associated with the right superior frontal gyrus (cluster 6) from the full sample ALE meta-analysis. Brain region label, hemisphere, MNI coordinates, ALE value, and cluster size (mm3).

Brain Regions	Hemi	x	y	z	ALE max.	Cluster Number	Volume (mm3)
Inferior frontal gyrus	L	-36	24	-6	0.45	1	111880
Inferior frontal gyrus	R	38	24	-8	0.23	2	17616
Posterior insula	R	40	-16	16	0.21	3	9448
Posterior cingulate/retrosplenial cortex	L	-8	-56	14	0.25	4	9360
Occipital pole	L	-12	-92	0	0.27	5	8176
Temporal occipital fusiform gyrus	L	-38	-56	-16	0.11	6	5000
Inferior lateral occipital cortex	R	42	-72	-10	0.11	7	3872
Amygdala	L	-20	-8	-18	0.16	8	3840
Superior lateral occipital cortex	L	-42	-74	34	0.10	9	3584
Middle temporal gyrus	L	-56	-42	6	0.07	10	3152
Lateral occipital cortex	L	-26	-64	50	0.12	11	3016
Amygdala	R	22	-6	-20	0.15	12	2320
Occipital pole	R	20	-94	0	0.10	13	2288

Note. L, Left; R, Right. MACM-cluster thresholded at $p<0.001$ corrected for multiple comparisons using cluster-level FWE correction at $p<0.05$. x, y, z coordinates provided in MNI space.

Table 2.S10: Cognitive decoding of full sample ALE meta-analysis using Neurosynth.Cognitive terms with the strongest associations with the pattern of activation foci for the full sample ALE results (top 20 terms with non-functional terms removed; see Methods for details on term selection).

Cognitive Term	r
social	0.18
Autobiographical	0.16
mental states	0.15
theory mind	0.14
mind	0.14
referential	0.14
self referential	0.14
value	0.13
reward	0.12
mentalizing	0.12
pain	0.12
autobiographical memory	0.12
painful	0.11
remembering	0.11
affective	0.10
memories	0.10
valence	0.09
comprehension	0.09
emotional	0.09
episodic	0.09

Chapter 3

Loneliness and meaning in life are reflected in the intrinsic network architecture of the brain

Mwilambwe-Tshilobo, L., Ge, T., Chong, M., Ferguson, M. A., Misic, B., Burrow, A. L., Leahy, R. M., & Spreng, R. N. (2019). Loneliness and meaning in life are reflected in the intrinsic network architecture of the brain. *Social cognitive and affective neuroscience*, 14(4), 423–433. https://doi.org/10.1093/scan/nsz021

3.1 Preface

The previous chapter used quantitative meta-analytic methods to examine the neural correlates of *state* loneliness. It showed that the neural response to the experience of social exclusion engaged distributed brain regions whose coactivation pattern overlapped with the topography of the default network — a neurocognitive network associated with cognitive processes that require internally-directed mentation. The study described in the current chapter examines whether *trait* loneliness is related to differences in the functional organization of brain networks that support internally and externally directed neurocog-

nitive processes. This work was published two years after the first resting-state fMRI study on loneliness. Layden et al. (2017) showed that loneliness was associated with changes in connectivity strength within and between two externally-directed brain networks that support both the maintenance and flexible control of attention. Their findings are consistent with the theoretical account of *trait* loneliness. They suggest that a putative mechanism characterized by diminished top-down control of attention may contribute to neurocognitive changes that orient attentional focus externally towards the social world.

However, as described in Chapter 1, the functional organization of the brain differs across individuals and differences in resting-state functional connectivity are meaningful predictors of behavior. To capture these individual differences in the intrinsic functional connectivity of the brain, we implemented a whole-brain individual connectome-based approach. Instead of focusing on select networks as in Layden et al. (2017) using a group-average parcellation approach, which has been shown to obscure individual-specific network organization related to behavior (Chong et al., 2017; Kong et al., 2015), we used an individualized parcellation method. This study aimed to establish a methodological proof of concept for taking a connectome-based approach to investigate individual differences in whole-brain network functional connectivity underlying loneliness. I applied this methodological approach to identify differences in the intrinsic functional organization of the brain that reflect individual differences in sociality in young adults. Here, we focused on the relationship between two well-established interdependent behavioral factors: loneliness and meaning in life.

3.2 Abstract

Social relationships imbue life with meaning, whereas loneliness diminishes one's sense of meaning in life. Yet the extent of interdependence between these psychological constructs remains poorly understood. We took a multivariate network approach to examine resting-state fMRI functional connectivity's association with loneliness and meaning

in a large cohort of adults (N = 942). Loneliness and meaning in life were negatively correlated with one another. In their relationship with individually parcelled whole-brain measures of functional connectivity, a significant and reliable pattern was observed. Greater loneliness was associated with dense, and less modular, connections between default, frontoparietal, attention and perceptual networks. A greater sense of life meaning was associated with increased, and more modular, connectivity between default and limbic networks. Low loneliness was associated with more modular brain connectivity, and lower life meaning was associated with higher between-network connectivity. These findings advance our understanding of loneliness and life meaning as distinct, yet interdependent, features of sociality. The results highlight a potential role of the default network as a central hub, providing a putative neural mechanism for shifting between feelings of isolation and purpose.

3.3 Introduction

Loneliness and life meaning are psychologically-bound constructs closely tied to sociality (Lambert et al., 2013; Stillman et al., 2009; Twenge et al., 2003). As a social species, humans typically seek out social bonds and search for meaning and purpose throughout the life-course. Indeed, both loneliness and a reduced sense of meaning are closely associated with declines in functional capacity (Perissinotto et al., 2012), dementia onset (Boyle et al., 2012; Holwerda et al., 2014), and mortality in later life (Boyle et al., 2009; Hill & Turiano, 2014; Holt-Lunstad et al., 2015). Despite these psychological and functional relationships, loneliness and meaning in life (MIL) are considered to be distinct constructs and their degree of interdependence remains poorly understood. Loneliness reduces the perception of a meaningful existence (Stillman et al., 2009)—the sense that life has purpose, significance, and coherence (Martela & Steger, 2016). This association appears to be reciprocal as MIL is strongly associated with the presence of close relationships (Ebersole, 1998; Klinger, 1977), and previous reports show that the subjective perception of a meaningful

life promotes social engagement and helps sustain close social bonds (**steptoe2013a**; Lambert et al., 2013). Loneliness arises due to deficiencies in the quality or quantity of social ties and the absence of social connectedness, in turn, diminishes MIL, suggesting that this relationship may also be reinforcing (Baumeister & Leary, 1995). But are these constructs opposite sides of the same coin, or are they emergent from distinct mechanisms?

Loneliness is characterized by implicit hyper-vigilance for social threats (S. Cacioppo et al., 2016). While this can facilitate the identification of viable social partners and prevent rejection, prolonged loneliness shifts exogenous attentional processes towards perceived social threats (Bangee et al., 2014; S. Cacioppo et al., 2015). Altered attention to external stimuli may affect how individuals internalize perceived information and make endogenous judgments about MIL (Hicks et al., 2010). Externally-and internally-guided cognitive processes are mediated by different neural networks and their interactions (Corbetta & Shulman, 2002; Spreng et al., 2010). This raises the possibility that loneliness and MIL are dissociable at the level of the brain, and subserved by distinct brain networks. Investigating how individual differences in loneliness and MIL are reflected within these neurocognitive systems may advance our understanding of their interdependence, and how they interact to guide adaptive and maladaptive behaviors.

A growing body of neuroimaging studies have provided important insights into the neural correlates of loneliness, reflecting changes in brain regions associated with processing of social information. In a task-based functional magnetic resonance imaging (fMRI) study, lonely individuals showed increased bilateral activation in the visual cortex in response to unpleasant social images compared to unpleasant non-social images. Regions implicated in reward processing (e.g. ventral striatum, amygdala) and perspective-taking (e.g. temporoparietal junction) showed lower activation when positive social images were presented, suggesting that lonely individuals may derive less pleasure from rewarding social stimuli (J. Cacioppo & Hawkley, 2009). Furthermore, other studies have linked loneliness to changes in brain morphology within the default network (DN), a neural system involved in social and self-related processes (Andrews-Hanna et al., 2014).

Loneliness is negatively correlated with grey matter volume (Kanai et al., 2012) and white matter density (Nakagawa et al., 2015) in the left posterior superior temporal sulcus (pSTS). These findings indicate that loneliness may compromise the structural and functional integrity of multiple brain regions.

Resting-state functional connectivity (RSFC) has been an invaluable analytic approach for investigating the functional interactions between anatomically separate brain regions and their relationship with behavior (Stevens & Spreng, 2014). Unlike task-based fMRI paradigms, resting-state functional magnetic resonance imaging (rs–fMRI) is task-free and can be used to simultaneously identify multiple functional networks correlated with behavior. Furthermore, previous analyses of rs-fMRI data from healthy adult populations have consistently shown strong congruence between brain networks derived from resting-state and those from task-based studies (Cole et al., 2014; Stevens & Spreng, 2014; Tavor et al., 2016).

Prior studies have used rs-fMRI to characterize intrinsic functional brain networks related to loneliness and MIL. Greater feelings of loneliness have been associated with less integrated connectivity between attention networks (Tian et al., 2017), as well as increased RSFC within the cingulo-opercular network, which is implicated in cognitive control (Layden et al., 2017). These intrinsic changes are consistent with behavioral reports of associations between hyper-vigilance and loneliness (S. Cacioppo et al., 2016). An investigation of the neural basis of meaning (Waytz et al., 2015) reported increased connectivity among regions of the medial temporal lobe subsystem of the DN, implicated in autobiographical remembering and mental simulation (Andrews-Hanna et al., 2014). While loneliness and MIL are correlated at the level of behavior, the analytical approaches used to characterize the neural representation of each construct have focused on functional connectivity of select brain regions or networks of interest, thus precluding inferences on a whole-brain level of integrated networks that can provide insight regarding the relationship between loneliness and MIL. Here, we investigate individual differences in the neural representation of loneliness and MIL within a single analytical framework.

The goal of the present study was to assess how whole-brain RSFC is associated with individual differences in loneliness and MIL. We characterized the intrinsic architecture of brain connectivity within a large population of healthy young adults using RSFC and individually parcellated brain regions (Chong et al., 2017), respecting that the localized topology varies across individuals in the cortex (e.g. Stevens et al., 2015) in order to identify the pattern of functional connectivity within and between large-scale networks. Using multivariate partial least squares (PLS), we characterized how patterns of RSFC relate to individual differences in perceived loneliness and MIL. This approach permits both replication of previous RSFC patterns, and exploratory examination of behavioral associations outside previously examined networks.

By examining the intrinsic functional connectivity underlying individual differences in loneliness and MIL, we test two hypotheses: First, loneliness would be associated with greater connectivity between regions that support attention, including the FPN, dorsal attention (DAN), and the ventral attention networks (VAN; (Corbetta & Shulman, 2002)). In contrast, MIL would be associated with greater connectivity within the DN. Our second hypothesis was that these patterns of RSFC would be inversely related (i.e. individuals with high levels of loneliness will share the same pattern of brain connectivity as those with a low sense of MIL and vice-versa). If confirmed, this would provide support for theoretical models of sociality suggesting that loneliness and MIL are distinct yet interdependent constructs (Lambert et al., 2013).

3.4 Methods

3.4.1 Participants

Participant data were collected as part of the Human Connectome Project (HCP) 1200 subject release dataset (http://www.humanconnectome.org). Participants were excluded if they did not meet the following criteria: (i) completed all rs-fMRI scans (REST1 and REST2); (ii) completed all relevant neuropsychological testing for emotional well-being;

(iii) participants with a score of 26 or below on the Mini Mental Status Examination (MMSE)—which could indicate marked cognitive impairments. Investigations of individual differences require large samples for adequately powered analyses. Assuming a typical correlation of approximately .25 between brain and behavior (e.g. (Hemphill, 2003), a sample of more than 120 is recommended in order to have 95% confidence that a correlation is greater than zero. A total of 942 healthy adults were included in the current study (53% female; mean age: 28.04; age range: 22–37). Table 1 shows the sample demographics.

Table 3.1: Sample Demographics

Gender	n	%	
Female	506	53.7	
Male	436	46.3	
Variable	Mean	s.d.	Range
Age	28.04	3.45	23–37
Loneliness	50.97	8.51	37.6–82.9
Meaning & Purpose	51.91	8.73	29.4–71.6
MMSE	29.05	0.99	23–30
Neuroticism	16.42	7.34	0–43
Extroversion	30.73	6.04	10–47
Agreeableness	32.12	4.95	13–45
Conscientiousness	34.56	5.91	11–48
Openness	28.33	6.26	10–47
Positive Affect	50.22	7.83	21.9–71.6

3.4.2 Behavioral measures

Behavioral assessments of social relationships and psychological well-being in the HCP sample included were obtained using the unadjusted scaled scores (t-scores) from the NIH Toolbox Emotion measures (http://www.nihtoolbox.org). All behavioral measures were treated as a continuous variable, and any references to high or low scores made are based on our specific sampling distribution.

Assessment of loneliness

Loneliness was assessed using the Loneliness survey from the NIH Toolbox on Emotion. This 5-item questionnaire is composed of items taken from a psychometrically validated assessment of loneliness (Salsman et al., 2013). Participants were presented with statements such as 'I feel alone and apart from others' (1 = Never; 2 = Rarely; 3 = Sometimes; 4 = Usually; 5 = Always).

Assessment of meaning in life

MIL was assess using the Meaning and Purpose survey from the NIH Toolbox on Emotion. This 18-item questionnaire is composed of items taken from psychometrically validated assessments of meaning and purpose (Salsman et al., 2013), and examines the extent to which people feel like their lives matter and make sense. An example item is, 'I have a good sense of what makes my life meaningful' (1 = Strongly disagree; 2 = Disagree; 3 = Neither agree nor disagree; 4 = Agree; 5 = Strongly agree).

Assessment of personality and positive affect

Neuroticism and extroversion have been previously shown to mediate the relationship between loneliness and dorsolateral prefrontal cortex (Kong et al., 2015), whereas personality and positive affect influence people's perception of MIL (King et al., 2006). Therefore, to assure the specificity of our findings, we controlled for these select covariates during our analysis. Personality measures of neuroticism, extroversion, openness, agreeableness, conscientiousness, were assessed using the 60-item version of the NEO-Five Factor Inventory. The NIH Toolbox Positive Affect Survey was used to assess participants' levels of positive affect during the past seven days. Participants were presented with statements such as 'I feel cheerful' (1 = Not at all; 2 = A little bit; 3 = Somewhat; 4 = Quite a bit; 5 = Very much).

3.4.3 Resting-state functional connectivity

The rs-fMRI data from the HCP was used for this study. The rs-fMRI runs were acquired for a total of 1 hour over the course of two sessions. For more details of the scan parameters, see Smith et al., (2013). Scans were processed using the HCP minimal preprocessing pipeline, which includes normalization to the MNI-152 template (Glasser et al., 2016). FIX ICA cleaned data was used for analysis (Glasser et al., 2016).

To identify functional networks, we parcellated the cortex into 400 functionally-defined regions for each individual separately. We refined the initial group parcellation developed by Schaefer et al., (2018) so that for each subject the parcel boundaries are optimized with respect to that subject's rs-fMRI (Chong et al., 2017). Initialization with a common parcellation results in automatic correspondence between parcels across subjects. By using a group sparsity constraint to model connectivity, we leveraged group similarities in connectivity between parcels while optimizing their boundaries for each individual. We applied this approach with initialization across the entire cohort in groups of 20 unrelated participants. Prior work on validating this approach showed improved homogeneity of resting activity within the refined parcels (Chong et al., 2017). Additionally, comparisons with task-based localizers showed a consistent reduction of variance of statistical parametric maps within the refined parcels relative to the group-based initialization indicating improved delineation of regions of functional specialization. This method enables a more accurate estimation of individual functional areas while maintaining consistency across individuals with a standardized topological atlas (Chong et al., 2017)). Each parcel was matched to a corresponding network in the 7 network parcellation by Yeo et al., (2011), which consisted of the visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default networks. For each participant, BOLD time-series for the two 15-min rs-fMRI scans within each session were temporally standardized (subtracted the mean and divided by standard deviation) and concatenated. The Pearson correlation coefficient between each pair of vertices was computed. The correlation coefficient matrix was then spatially standardized and averaged within and across parcels, resulting in a 400 x 400 functional connectivity matrix (Ge et al., 2017). The two connectivity matrices computed from the two sessions for each participant were averaged.

3.4.4 Behavioral data analysis

Three sets of analysis were performed to examine the behavioral relationship between loneliness and MIL in Python (https://www.python.org/). In our first analysis, we used

the t-scores for the self-report behavioral measures and calculated the Pearson correlation coefficient between each measures. This also allowed us to determine whether loneliness and MIL were inversely related to one another using the NIH-emotion scales. We also examined this association controlling for covariates (age, gender, MMSE, positive affect, and personality measures) using partial correlation. Finally, t-tests were conducted to identify possible gender differences in the distribution of scores between loneliness and MIL, as well as in covariates of interest. Statistical significance was set at $P < 0.05$.

3.4.5 Partial Least Squares analysis

PLS was performed to quantify RSFC related to individual differences in loneliness and MIL. PLS is a multivariate statistical technique which uses a data-driven approach to directly measure brain-behavior relationships (McIntosh & Lobaugh, 2004; McIntosh & Mišić, 2013). We chose this method of analysis because it allowed for inferences about individual differences in the intrinsic connectivity of large–scale neurocognitive networks. PLS identifies linear combinations of the original variables (functional connections and behavioral measures) that maximally covary with each other across participants. The resulting patterns (termed latent variables or LVs) can be interpreted as optimally-paired functional networks and behavioral phenotypes, respectively.

In the present study, we used PLS to examine the relationship between RSFC, loneliness, and MIL. Two matrices were computed for this analysis. The X matrix was organized such that the parcellated functional connectivity matrix for each participant was concatenated, resulting in a 942 x 400 x 400 matrix. The Y matrix consisted of the individual scores for loneliness and MIL for all participants, creating a 942 x 2 matrix. The X and Y matrix were centered and normalized across participants. Singular value decomposition of the cross-correlation matrix X'Y yields several mutually-orthogonal LVs, each composed of three elements: (i) a left singular vector, containing weights for each of the behavioral measures; (ii) a right singular vector, containing weights for each of the functional connections; and (iii) a scalar singular value. Squared singular values reflect effect

size: they are proportional to the covariance between connectivity and behavior that is accounted for by each latent variable. The number of latent variables is equal to the rank of X'Y; in the present case, this is the number of behavioral measures (ii).

The significance and reliability of each LV were evaluated in permutation testing and bootstrap resampling, respectively. We first assessed the significance of the pattern of functional connectivity captured by a given LV using permutation tests to determine how different the results are from chance. To do this, 500 permutation tests were computed in which the order of the rows of one of the data matrices (X) was randomly rearranged. Columns of the permuted matrix are then correlated with the behavioral matrix Y and the correlation matrix is subjected to singular value decomposition as described above. This process generates a distribution of singular values under the null hypothesis that there is no relationship between functional connectivity and behavior. The significance of the LV is estimated by computing the proportion of times the permuted singular values (covariance explained) is higher than the observed singular values (significance thresholded at $P < .05$).

To assess the reliability of weights for individual connections and behavioral measures, we used bootstrap resampling. The rows of both data matrices (X and Y) were sampled with replacement and a resampled correlation matrix (X'Y) was re-computed. The matrix was subjected to singular value decomposition and the process was repeated 500 times to estimate a sampling distribution for each singular vector (i.e. connection and behavior) weight. To identify connections and behaviors that (a) make a large contribution to the overall multivariate pattern and (b) are relatively insensitive as to who is in the sample, we calculated the ratio between each weight and its bootstrap-estimated standard error. The resulting 'bootstrap ratios' (BSRs) are large for connections/behaviors that have large weights and narrow confidence intervals. If the sampling distribution is approximately unit normal, BSRs are equivalent to z-scores. Brain network connections were considered reliable if the absolute value of the BSR > 2 (approximately $P < .05$) and were visualized using BrainNet Viewer (Xia et al., 2013). To account for potential con-

founds, multiple regression analysis was performed on the brain connectivity scores with behavioral scores controlling for age, gender, personality measures, and positive affect.

We also examined the extent to which network-level functional connectivity contributes to individual differences in behavior. To quantify the network-level contributions to the connectivity pattern identified by the PLS analysis, two separate weighted adjacency matrices were constructed reflecting the positive and negative PLS weights, respectively. The nodes of the graph represent the 400 brain regions defined by the individual parcellation scheme, and the edges represent the BSR weight for each pairwise connection. The matrices were thresholded such that BSRs with an absolute value less than 2 were set to 0. Positive BSRs greater than 2 were set to 1, and negative BSRs less than −2 were set to −1. The network-level functional connectivity contributions were quantified by averaging the weights of all connections in a given network, thus generating a 7 x 7 matrix. Next, permutation testing was applied on the full thresholded matrix by randomly re-ordering the network labels (preserving the number of nodes originally assigned to each network) and re-calculating the network means 1000 times to build a sampling distribution under the null that network assignment does not contribute to the connectivity pattern. The significance of the pairwise connections of the original 7 x 7 matrix was determined by estimating the proportion of times the values of the sampling distribution were greater than or equal to the original value (Shafiei et al., 2019).

3.4.6 Modularity analysis

To further characterize the pattern of connectivity identified by the PLS analysis, we quantified modularity, a global network measure that estimates how well a network can be divided into modules (or communities) with stronger within-module than between-module connections (Girvan & Newman, 2002). Modular organization within a network is as a metric of efficient information processing and relates to functional specialization (Bullmore and Sporns, 2009). The modularity measure Q(p) for a given partition p of a graph G can be defined as the proportion of edges in G, that fall within the same module, sub-

tracted from the proportion of edges that would be expected by chance. The objective of this modular algorithm is to identify the partition p that maximizes Q. A modularity value of $Q = 0$ is expected if the edges of a graph were formed randomly, while a graph with a $Q > 0.3$ is generally an indicator of significant modular structure (Newman & Girvan, 2004). There are multiple methods for identifying modules, however, and here we used an a priori mapping of nodes to the network modules defined by Yeo et al., 2011. This allowed us to quantify the strength of segregation of functional networks. We sub-divided the thresholded PLS connectivity matrix into two separate graphs: one containing just positive PLS weights and the other the negative PLS weights. Graph theoretical analyses were performed using functions implemented using the Brain Connectivity Toolbox (Robinson et al., 2010). Network modularity estimates were computed with a Louvain-like fast-unfolding algorithm (**Blondel˙2008**), using the average modularity across 1000 runs of the algorithm.

3.5 Results

3.5.1 Descriptive data analysis

Sample characteristics for age, gender, loneliness scores, meaning in life scores, MMSE scores, personality scores, and positive affect are displayed in Table 3.1. Pearson correlation between these behavioral measures revealed a negative correlation between loneliness and meaning in life ($r(940) = -.45$, $P < .001$, 95% CI $= [0.53, 0.36]$), which supports previous findings (Stillman et al., 2009). Loneliness was also negatively correlated with extroversion ($r(940) = -.42$, $P < .001$, 95% CI $= [-0.50, -0.32]$), agreeableness ($r(940) = -.27$, $P < .001$, 95% CI $= [-0.36, -0.17]$), and conscientiousness ($r(940) = -.32$, $P < .001$, 95% CI $= [-0.41, -0.22]$), and positive affect ($r(940) = -.47$, $P < .001$, 95% CI $= [-0.55, -0.39]$); and positively correlated with neuroticism ($r(940) = .57$, $P < .001$, 95% CI $= [0.49, 0.64]$) and openness ($r(940) = .08$, $P = .01$, 95% CI $= [-.02, 0.18]$). MIL was negatively correlated with neuroticism ($r(940) = -.43$, $P < .001$,95% CI $= [-0.51, -0.34]$) and gender

(r(940) = −.09, P = .01, 95% CI = [−0.19, 0.01]), and positively correlated with extroversion (r(940) = .40, P < .001, 95% CI = [0.31, 0.49]), agreeableness (r(940) = .25, P < .001, 95% CI = [0.15, 0.34]), conscientiousness (r(940) = .35, P < .001, 95% CI = [0.26, 0.44]), and positive affect (r(940) = .52, P < .001, 95% CI = [0.43, 0.59]). No other significant correlations were noted between covariates.

Analyses were also conducted to determine any gender differences in behavioral measures. The means and standard deviations for loneliness, MIL, personality traits, and positive affect by gender are displayed in Supplementary Table 3.S1. While there was no significant gender differences for loneliness in our sample, t(940) = 0.34, P = .73, d = 0.02, female participants reported higher meaning in life scores (M = 52.62, s.d. = 8.69) than male participants (M = 51.09, s.d.= 8.71), t(940) = 2.70, P < .01, d = 0.18).

3.5.2 Intrinsic functional connectivity results

We first examined the multivariate relationship between RSFC, loneliness, and MIL using behavioral PLS. The analysis identified one significant pattern of connectivity that reliably expressed individual differences in loneliness and MIL (loneliness r = −.10; MIL r = .13; permuted P = .01; 17.6% covariance explained). Loneliness was found to negatively correlate with the pattern of brain connectivity of LV1, whereas MIL correlated positively with this pattern of brain connectivity (Figure 3.1C). To assure the specificity of these results, a partial correlation analysis was used to test whether the relationship between behavioral measures and brain connectivity scores remained significant after controlling for age, gender, personality, and positive affect. The results remained significant for loneliness (pr(932) = −.08; P = .01, 95% CI [−0.14, −0.01]) and MIL (pr(932) = .09, P = .003, 95% CI [0.03, 0.16]).

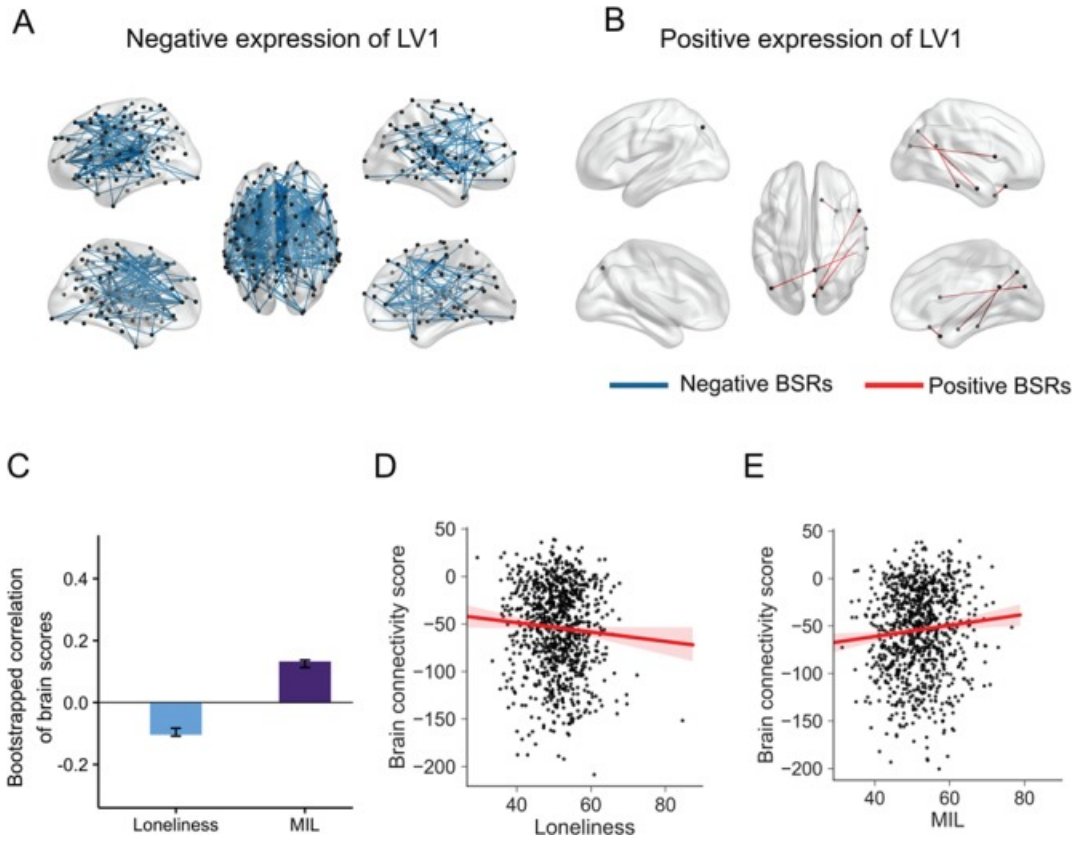


Figure 3.1: Analysis revealed one significant latent variable (LV). The functional connections that most reliably express the brain/behavior correlations thresholded at 95% bootstrap ratio. The pattern of connectivity for LV1 depicted in (A) blue represent the connectivity weights for LV1 that covary negatively with loneliness, while those in (B) red covary positively with meaning in life (MIL). The top 2% connections are shown for each. (C) Correlations between participants' brain connectivity scores and behavioral measures for LV1. Error bars indicate the 95% confidence intervals derived from the bootstrap estimate. Scatter plots show the relationship captured by the PLS analysis for individual brain connectivity scores corrected for age, gender, positive affect, and personality measures as a function of loneliness (D) and MIL (E).

Figure 3.1A-B shows reliable ROIs that covary with each other. The edges connecting the nodes for the negative and positive dimension of LV 1 represent the top 2% BSR weights. Overall, the connectivity pattern for the negative expression of LV 1 showed densely interconnected nodes when compared to that of the positive expression. Participants with high levels of loneliness showed extensive between-network connectivity

across the brain. Specifically, nodes located within DN, SOM, and FPN were highly interconnected. In addition, functional connectivity was observed between bilateral regions in the visual network with the frontal and parietal operculum. In contrast, high levels of MIL correlated with increased functional connectivity between regions involving the DN and limbic network. This included bilateral connectivity between posterior parietal regions, as well as with nodes located in the anterior regions of the FPN.

The PLS analysis identified reliable connectivity patterns that explain individual variability in loneliness and MIL. However, from the results of this analysis alone, it is difficult to gauge whether certain networks contribute more to the overall network connectivity pattern than others. To address this question, we used permutation testing on the functional covariance matrix representing the pairwise BSRs for each of the 400 brain regions (Figure 3.2A) to examine the relative within and between network contributions of the seven networks defined by the parcellation scheme. As shown in Figure 3.2B, the strongest contributions to the RSFC pattern associated with the negative expression of LV1 were from the DN and FPN. Specifically, between network connections of both the DN and FPN with VIS, SOM, and VAN were found to contribute significantly to the overall connectivity pattern (DN: VIS $=$ P $<$ .001; SOM $=$ P $<$ .001, and VAN $=$ P $<$ .05; FPN: VIS $=$ P $<$.001; SOM $=$ P $<$.001, and VAN $=$ P $<$.001). For MIL, we found that both between and within network connectivity contributed to the RSFC pattern (Figure 3.2C). The pairwise connections that contributed the strongest were between the DN with the LIM (P $<$ .001) and FPN (P $<$ 0.05); the FPN and the VIS (P $<$ .05) and LIM (P $=$ .001); and between the VIS and the DAN (P $<$ .05) and VAN (P $<$ .01). As for the within network connectivity, the DAN (P $<$ .05), VAN (P $<$ .05), LIM (P $<$ .001), and DN (P $<$ .01) were found to contribute significantly to the RSFC pattern related to MIL.

3.5.3 Gender control analysis

To account for possible effects of gender, an ANCOVA was conducted to examine the effects of gender on the PLS brain scores while controlling for age, personality, and pos-

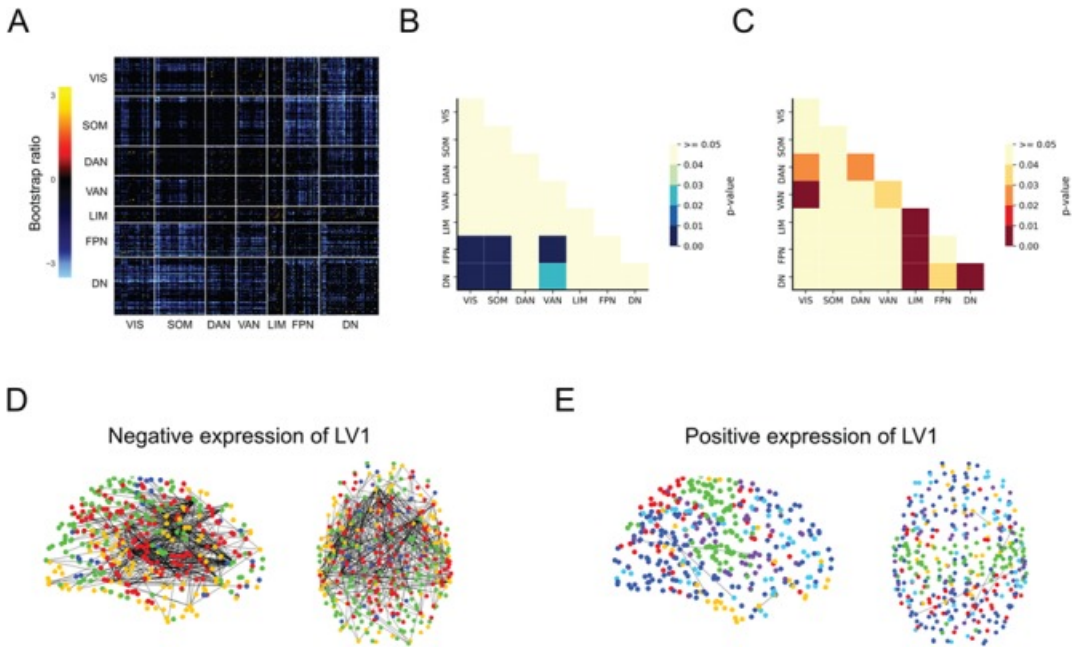


Figure 3.2: (A) The correlation matrix of reliable pairwise connections associated with loneliness and meaning in life (MIL; thresholded bootstrap ratio $\pm$ 2.0 to 3.5). Significant contributions of resting-state network pairs to the connectivity pattern for the (B) negative expression of the first latent variable (LV1) and (C) positive expression of LV1. Sagittal and axial views of the resting-state functional connectivity pattern associated (D) loneliness and (E) MIL. The colors indicate the nodes that belong to the same module and node size is proportional to the number of edges connecting it to the network. VIS = visual; SOM = somatomotor; DAN = dorsal attention; VAN= ventral attention, LIM = limbic, FPN = frontoparietal network; DN = default network.

itive affect. We found that there was a significant effect of gender, $F(1,933) = 30.48$, $P < .001$, partial eta squared = .032. We then reanalyzed the data to assess the relationship between RSFC, loneliness, MIL, and gender. In the group analysis using PLS, the brain-behavior correlation for both groups co-varied together. Critically, no gender interaction was observed (see Supplementary Figure 3.S1). This suggests that the magnitude of the association is weaker in women. However, the associations with functional connectivity are still significant when controlling for gender, in addition to neuroticism, extroversion, agreeableness, conscientiousness, openness, positive affect and age; for loneliness [$pr(932) = -.08$, $P < .05$] and MIL [$pr(932) = .10$, $P < .005$].

3.5.4 Modularity

Having established that cohesion within and between select networks appears to play an important role in the connectivity profiles underlying differences in behavior, we sought to investigate the global network organization of LV1 by assessing the modular structure of the connectivity pattern. The modularity for each pattern provides a metric for quantifying the segregation of functional networks, with higher Q indicating a stronger segregation of functional networks. Using a pre-defined partition based on the modules previously reported in (Yeo et al., 2011), we calculated the modularity quality index Q of the thresholded weighted graphs representing the negative and positive BSR weights. Graphical representations of the modular structure associated with each behavior are shown in Figure 3.2D-E (see Figure 3.3B-C for projections on the cortical surface). The features of community structure for loneliness and MIL differed in the number of communities detected and in the distinctiveness of these communities. While the algorithm used to examine the community structure revealed 7 modules for MIL that largely corresponded with the pre-defined partition, we identified only 5 modules for the connectivity pattern for loneliness. Specifically, nodes previously assigned to the FPN and DN appear to be integrated with parts within the SOM and VIS networks (Figure 3.3B). Next, we measured the mean Q to quantify the segregation of functional networks and found that loneliness was less segregated (mean Q $= 0.15$) relative to MIL (mean Q $= 0.58$). Taken together, these findings reflect that loneliness and MIL are characterized by differences in modular organization of brain networks.

3.6 Discussion

Loneliness and meaning in life are important for guiding everyday behavior and sustaining mental health and well-being over the life course and into advanced age. Yet their neural signatures remain poorly understood. Here we used a multivariate analytical model to examine patterns of intrinsic functional connectivity associated with individual

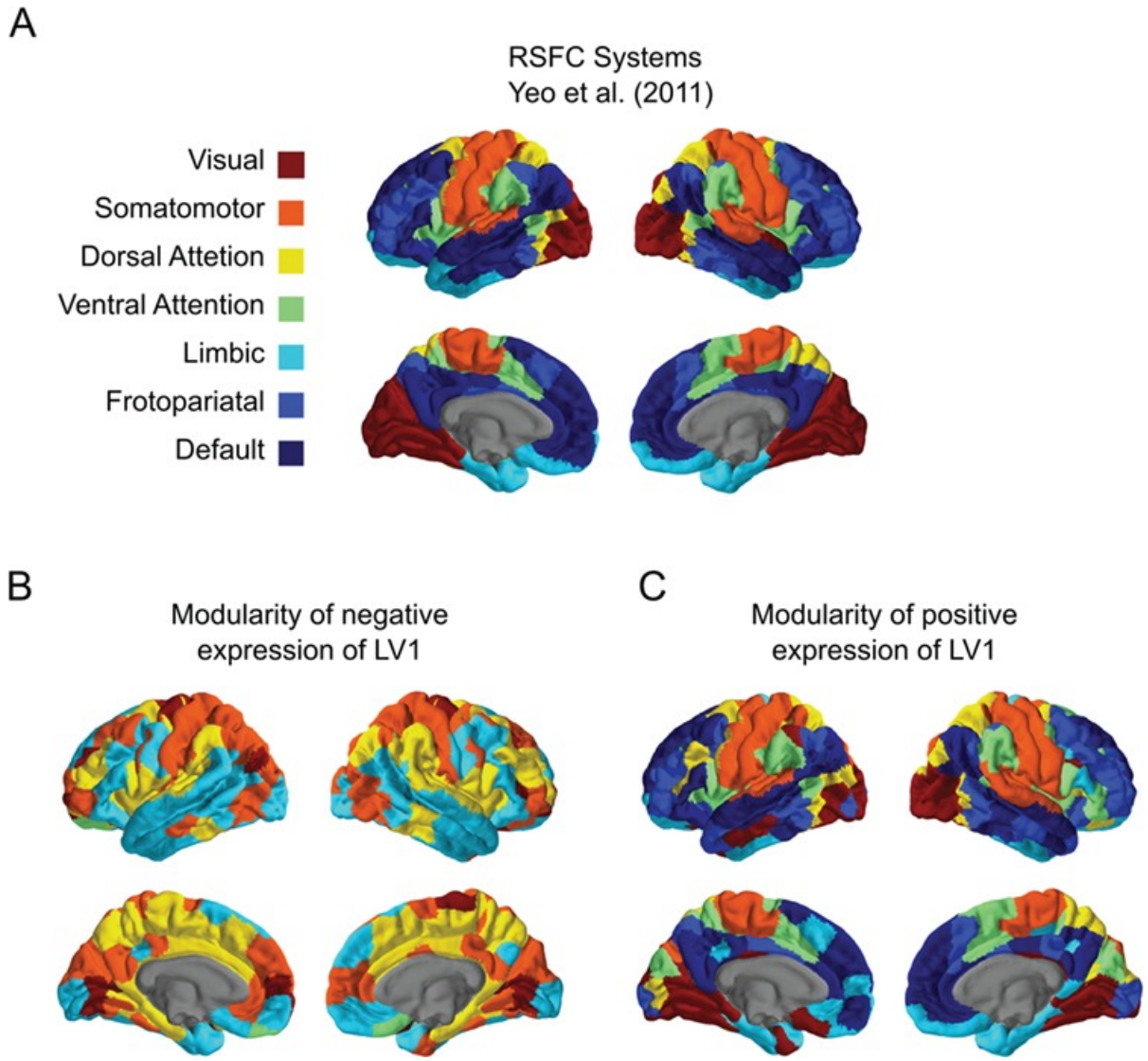


Figure 3.3: (a) the modular organization for RSFC defined by 7 network parcellation of Yeo et al., (2011). Modular organization of the connectivity pattern associated with (b) high-loneliness/low-meaning in life and (c) high-meaning in life/low-loneliness. Color-coding brain regions according the module assignment in (a).

variability in loneliness and MIL in a large sample of healthy adults. There were three primary findings. First, we identified reliable patterns dissociating whole-brain RSFC related to individual differences in loneliness and MIL. Second, we observed a core role for default network connectivity in differentiating loneliness and meaning in life. While default and frontoparietal interactions, among others, were associated with higher levels

of loneliness, this pattern differed for MIL where connectivity between default and limbic brain regions was associated with a greater sense of meaning. Finally, greater feelings of loneliness were associated with lower modularity, or increased integration, between the default and frontoparietal networks and more externally-oriented networks including somatosensory and visual brain regions. In contrast, a stronger sense of life meaning was associated with greater modularity among the limbic and default networks.

Current theoretical models of sociality suggest that loneliness and MIL are discrete yet interdependent, and potentially reinforcing (Lambert et al., 2013; Stillman et al., 2009; Twenge et al., 2003). However, only a few studies have investigated the relationship between the loss of social functioning (i.e. loneliness) and MIL, and these have primarily employed behavioral methods (Lambert et al., 2013; Stillman & Lambert, 2013). More recently, investigations into the intrinsic functional architecture of the brain at rest (i.e. in the absence of explicit task demands) have demonstrated that these durable features of brain organization can enhance our understanding of enduring features of mental function (Smith et al., 2015; Stevens & Spreng, 2014). Here we leveraged this idea to explore patterns of functional connectivity associated with individual differences in loneliness and MIL.

We predicted that the DN, through its role in mediating internally directed cognition, would be associated with MIL. A greater sense of life meaning has previously been associated with increased connectivity within the medial temporal lobe subsystem of the DN (Waytz et al., 2015). Our data complements this finding by showing increased connectivity within nodes of the DN associated with higher MIL. Additionally, we observed a robust, albeit unpredicted, pattern of connectivity within and between networks typically implicated in internally-directed cognitive processes associated with higher MIL, including the limbic and default networks, as well cognitive control regions of the FPN. The limbic network is involved in emotional processing, which involves monitoring, evaluating, and adjusting emotional reaction to align with current goals. Thus the ability to internally reflect upon one's affective state, may be important for a sense of meaning, par-

ticularly when experiencing negative emotions (Kross et al., 2011). Consistent with this idea, individuals with a clear sense of purpose in life report lower levels of negative affect and less emotional reactivity to stressors in daily life (Hill et al., 2018).

The evolutionary theory of loneliness posits that feeling lonely is an aversive biological signal that motivates the individual to repair or seek new social relationships, and leads to neural changes that impact attention and processing of social information (J. Cacioppo & Cacioppo, 2018). While our findings are in accordance with previous studies linking loneliness with altered RSFC in networks related to attention and executive control (Layden et al., 2017), the results point to broader changes in brain connectivity across multiple networks. As with MIL, the most robust associations were observed for between network interactions, and specifically between the DN and FPN as well as networks implicated in more externally-directed cognition including attentional (e.g. VAN) or perceptual (e.g. SOM and visual networks) processing. While the breadth of these associations was not predicted, the VAN is associated with bottom-up or externally monitoring for behaviorally salient features of the environment (Corbetta & Shulman, 2002), presumably detected through connections with these perceptual systems. While we are unable to directly confirm this with the current data, this is consistent with behavioral accounts of hyper-vigilance for external social threat associated with loneliness. Further, the DN has been implicated in low mood and ruminative thoughts (DuPre & Spreng, 2018), which may be elevated by a sense of loneliness. However, several methodological considerations may account for differences between Layden et al. (2017) and the current findings. While a whole-brain analytic approach was used in both, we examined connectivity strength using individually-parcellated neurocognitive networks—thereby accounting for inter-subject functional connectivity variability—rather than focusing on standardized network parcellation schemes. Further, we used multivariate, data-driven analytical methods and a single model approach, including MIL whereas the earlier study focused on attention networks to test their hypotheses. Further, PLS methods allow for identification of both within and between network connectivity strengths in a single ana-

lytical model (McIntosh & Lobaugh, 2004; McIntosh & Mišić, 2013). Here, the between network associations were among the most robust, and most discriminating, patterns observed for loneliness and life meaning.

Our second hypothesis was based in part on recent findings that individual differences in both positive and negative behavioral traits have been associated with a unique configuration of intrinsic functional connectivity (Smith et al., 2015). Specifically, increased connectivity within regions encompassing the DN was linked to positive behavioral traits such as life satisfaction, and inversely related to negative behavioral traits such as perceived stress (Smith et al., 2015). Similarly, by including both loneliness and MIL in a single model, here we were able to identify a single pattern of functional connectivity implicating the DN that was associated with these positive and negative constructs. Connectivity within the DN, and its connections to the limbic network, were associated with a higher sense of life meaning and lower feelings of loneliness. In contrast, DN connectivity to externally-oriented attentional systems and cognitive control networks was associated with a higher sense of loneliness, and lower life meaning.

We further examined the features of whole-brain RSFC organization related to loneliness and MIL by interrogating the modular intrinsic network architecture. Increased modularity has been associated with more efficient processing operations and is generally considered to be a marker of brain health (Bullmore & Sporns, 2009; Wig, 2017). The intrinsic network organization of brain networks associated with loneliness was less modular as the DN and FPN were less differentiated from externally-directed attention and perceptual networks. As suggested above, this pattern of network dedifferentiation may reflect increased vigilance for social threat. Consistent with this idea, less modular brain network architecture has been associated with negative affect including depression, as well as normal and pathological aging (Andrews-Hanna et al., 2014).

To our knowledge, this is the first study to investigate whole-brain patterns of RSFC associated with loneliness and MIL. Both MIL and loneliness are predictors of successful aging and an important future direction would be to examine how these patterns of in-

trinsic brain networks change in normal and pathological aging. Future examinations will also be necessary to explore how the connectivity patterns identified in the present study are dynamically shaped in response to task demands that require judgments of belonging and/or existential meaning. Further, MIL is distinct from meaning-seeking and meaning maintenance, and these differences will need to be explored with respect to loneliness and patterns of RSFC. This question is particularly relevant in light of past work demonstrating a distinction between the presence of meaning and the search for meaning (Heine et al., 2006; Steger, 2012), and may have important implications for the interpretation of our results for loneliness given that the lack of belonging could both motivate or discourage an individual's search for meaning.

By investigating associations between brain function, loneliness and MIL within a common analytical framework, we were able to identify a pattern of intrinsic functional connectivity that differentiated brain networks associated with higher MIL and lower loneliness from those associated with lower MIL and higher loneliness. Critically, between network interactions, particularly those involving the DN, were among the most robust and discriminating intrinsic network markers of loneliness and MIL. Behaviorally, these findings advance our understanding of these two constructs as distinct, yet interdependent, features of sociality (Lambert et al., 2013; Stillman et al., 2009; Stillman & Lambert, 2013). While speculative, the data also implicate the DN as a candidate network hub, suggesting that these brain regions may provide a neural conduit for shifting between feelings of isolation and purpose. If confirmed, these findings may inform future research to design behavioral and neural intervention strategies targeted at disrupting the reinforcing cycle of loneliness and life meaning.

3.7 Conflict of interest

The authors declare no competing financial interests.

3.8 Acknowledgments

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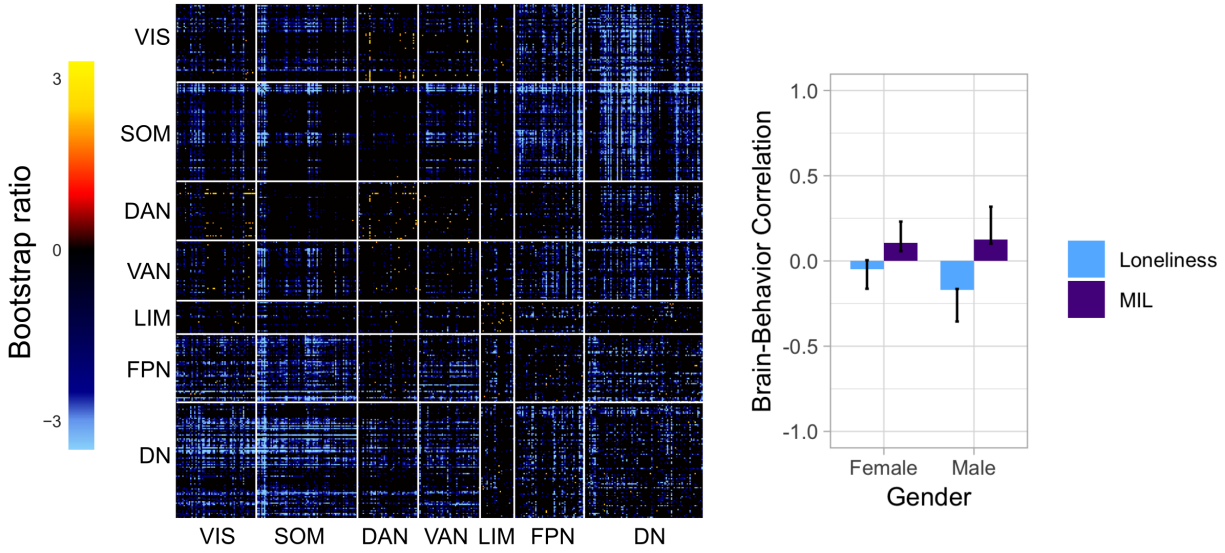
3.10 Supplementary Figures



Supplementary Figure 3.S1: The figure displays the results for the first latent variable which identified a pattern of connectivity that reliably expressed the relationship between loneliness and MIL (permuted p = .006; 25% covariance explained) in both female (loneliness r = -0.05; MIL r = 0.10) and male (loneliness r = -0.17; MIL r = 0.13) participants. The error bars represent the 95% bootstrapped confidence interval. The matrix is thresholded to ±2 to 3.5 bootstrap ratio.

3.11 Supplementary Tables

Table 3.S1: Means and SD of behavioral measures separated by gender

	Female	Male	
	(Mean$\pm SD$)	(Mean$\pm SD$)	t-test (t,p)
Loneliness	51.06$\pm$8.08	50.87$\pm$8.99	(0.34, .732)
Meaning & Purpose	52.62$\pm$8.69	51.09$\pm$8.71	(2.70, .007)**
Neuroticism	17.15$\pm$6.87	15.56$\pm$7.75	(3.33, .001) ***
Extroversion	30.75$\pm$5.93	30.71$\pm$6.15	(0.09, .925)
Agreeableness	32.97$\pm$4.64	31.13$\pm$5.11	(5.79, .001) ***
Conscientiousness	35.17$\pm$5.81	33.86$\pm$5.94	(3.43, .001) ***
Openness	27.80$\pm$6.04	28.94$\pm$6.44	(-2.81, .005) **
Positive Affect	50.44$\pm$7.85	49.96$\pm$7.81	(0.94, .346)

** p 0.01; *** p 0.001, two-tailed

Chapter 4

Age differences in functional brain networks associated with loneliness and empathy

Mwilambwe-Tshilobo,L., Setton, R., Bzdok, D., Turner, G. R., Spreng, R.N. (*in press*). Age differences in functional brain networks associated with loneliness and empathy. *Network Neuroscience*.

4.1 Preface

Age-related changes in the associations between sociality and brain function into late adulthood are not well understood. The previous chapter showed that in younger adults, individual differences in the functional connectivity between externally and internally-directed brain networks are related to *trait* loneliness. Interestingly, a separate study from our lab examining neural correlates of loneliness in a large middle-aged cohort reported that in middle adulthood, loneliness was associated with greater functional integration of the default network with limbic and frontoparietal control networks (Spreng et al., 2020). This divergence in RSFC-loneliness associations raised several questions regard-

ing the completeness of the current theoretical conceptualizations from an adult lifespan perspective. Age differences are often observed within- and between-network connectivity across the lifespan. Moreover, age-related shifts in socioemotional goals influence the types of relationships people seek to nurture and the social cognitive processes needed to support human sociality. Without directly comparing adults in early and middle/late adulthood, it is unclear whether the observed differences reflect a shift in the association between loneliness and RSFC.

4.2 Abstract

Loneliness is associated with differences in resting-state functional connectivity (RSFC) within and between large-scale networks in early and middle-aged adult cohorts. However, age-related changes in associations between sociality and brain function into late adulthood are not well understood. Here, we examined age differences in the association between two dimensions of sociality –loneliness and empathic responding– and RSFC of the cerebral cortex. Self-report measures of loneliness and empathic capacity were inversely related across the entire sample of younger (mean age = 22.6y, n=128) and older (mean age = 69.0y, n=92) adults. Using multivariate analyses of multi-echo fMRI RSFC, we identified distinct functional connectivity patterns for individual and age-group differences associated with loneliness and empathic responding. Loneliness in young and empathy in both age groups was related to greater visual network integration with association networks (e.g., default, frontoparietal control). In contrast, loneliness was positively related to within and between network integration of association networks for older adults. These results extend our previous findings in early and middle-aged cohorts, demonstrating that brain systems associated with loneliness, as well as empathy, differ into older age. Further, the findings suggest that these two aspects of social experience engage different neurocognitive processes across human lifespan development.

4.3 Author Summary

Feelings of loneliness emerge when a person's desire or need for interpersonal relationship is unmet. This state of perceived social isolation can influence social cognitive processes that are critical for connecting with others, such as empathy. Neuroimaging studies have shown diverging functional connectivity patterns among functional brain networks between lonely younger and middle-aged adults. Here, we take a targeted approach to directly assess age-related differences in functional connectivity associated with loneliness and empathic responding in younger and older adults. We find evidence that individual differences in functional connectivity related to loneliness and empathic responding differs with age. We discuss possible mechanisms underlying these associations and their implications for brain and social functioning across the adult lifespan.

4.4 Introduction

Forming and maintaining social bonds are among the most complex of human abilities. Sociality and the emergence of social collaboration within species have been linked to larger brain sizes, with humans at the peak of this evolutionary continuum (Dunbar & Shultz, 2007). Social functioning is related to functional activation and connectivity among multiple large-scale brain systems (Mars et al., 2012; Moran et al., 2012; Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020). The importance of sociality as a determinant of brain health is most evident when social needs go unmet. Perceived social isolation, or loneliness, has a significant negative impact on mental and physical health (S. Cacioppo et al., 2014; Ong et al., 2016; Shankar et al., 2013; Tilvis et al., 2011). Lonely individuals experience increased risk for cognitive decline (Boss et al., 2015), neuropathological burden (d'Oleire Uquillas et al., 2018; Donovan et al., 2016), and Alzheimer's disease (Wilson et al., 2007). Although loneliness is related to adverse cognitive sequelae in age-related brain disease, much of the research investigating the impact of loneliness on brain structure and function has been conducted in younger or middle-aged adults (see Lam et al.,

2021 for a review). Loneliness poses significant health risks and is a burden, particularly for older adults. However, its differential impact on brain function in early and late adulthood remains largely unexplored.

Although the experience of loneliness varies between people, it emerges because one's need for social connection is unfulfilled (J. Cacioppo & Hawkley, 2009). The felt absence of connection has marked effects on the cognitive and affective processing of social signals (Bangee et al., 2014; S. Cacioppo et al., 2015). Identifying these perceptual and attentional changes led to altered social functioning resulting from deficits in social perception ((J. Cacioppo & Hawkley, 2009). Poor perception of social cues associated with feeling lonely may hinder the ability to recognize and accurately interpret others' thoughts and feelings, both core features of empathic responding. Preliminary evidence in younger adults indicates that this interaction may alter the impact of loneliness on the brain. Loneliness is inversely related to white matter integrity in brain regions implicated in social-cognitive processes, with higher empathy moderating this relationship (Nakagawa et al., 2015). This finding suggests that the negative behavioral association between loneliness and empathy may have a direct neural correlate, with each exerting opposing brain effects.

A growing body of neuroimaging studies now link individual differences in loneliness (J. Cacioppo & Hawkley, 2009; Düzel et al., 2019; X. Kong et al., 2015; Layden et al., 2017; Nakagawa et al., 2015; Wong et al., 2016) and empathy (Schurz et al., 2021; Völlm et al., 2006) to structural and functional changes in brain regions spanning multiple neurocognitive systems. Resting-state functional connectivity (RSFC) has demonstrated that interactions among spatially distributed brain regions underlie individual differences in loneliness (Feng et al., 2019; Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020) and empathy (Christov-Moore et al., 2020; Katsumi et al., 2021).

However, many open questions remain regarding the neural associations between loneliness and empathy. First, empathy is a multidimensional construct consisting of cognitive and affective components, each with distinct neural patterns (Cox et al., 2012; Schurz et al., 2021). Although behavioral evidence suggests that associations with loneli-

ness vary in strength between components (Beadle et al., 2012), this finding may not extend to all aspects of empathic responding (i.e., empathic concern and perspective taking; Kanai et al., 2015). Second, associations between empathic responding and loneliness may change as people age. As people age, the quality of social relationships becomes more important than quantity ((L. Carstensen, 1992). Older adults experience higher risks for loneliness (i.e., social isolation; Steptoe, Shankar, Demakakos, Wardle, 2013; Luhmann Hawkley et al., 2016). Because loneliness impacts social perception (J. Cacioppo & Hawkley, 2009), lonely older adults may have more difficulties forming meaningful social bonds. Variability in the impact of aging on the neural correlates of cognitive and affective components of empathy (Beadle & de la Vega, 2019) may also influence age differences in the relationship between loneliness and empathic responding. Therefore, understanding the role aging plays in both behaviors may provide more insights into their individual and combined effects on the intrinsic functional connectivity of the brain.

Two studies investigating the relationship between RSFC and loneliness provide early evidence for putative age differences in brain-loneliness associations. In a large cohort of younger adults, Mwilambwe-Tshilobo et al. (2019) identified that loneliness was associated with greater RSFC between default network regions and visual and attention networks. These associations correlate with loneliness-related neural changes in externally-directed perceptual and attention networks (Schurz et al., 2021) and support the altered social perception hypothesis of loneliness and empathy (S. Cacioppo et al., 2015). In contrast, a population-based study of late middle-aged adults revealed that loneliness was positively related to RSFC between the default network and frontoparietal control and limbic regions, but not the visual network (Spreng et al., 2020). In addition, while higher default network integration was negatively correlated with loneliness in young adults (Mwilambwe-Tshilobo et al., 2019), it was positively associated with loneliness in middle-aged adults. The authors provided evidence that loneliness in middle age may precipitate more internally-directed cognitive processes, mediated by the default network, as lonely individuals mentalize about desired but unmet social interactions. Combined, these two

studies suggest a shift in the impact of loneliness from changes occurring in externally-directed neurocognitive systems in early adulthood, to an upregulation in brain networks associated with internally-directed mental processes in middle adulthood.

The relationship between loneliness and age is U-shaped, with peaks at 30 and 60 years of age (Luhmann & Hawkley, 2016). This relationship corresponds to the average ages in the younger and middle-aged studies described above (Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020), raising the intriguing possibility that, while the prevalence of loneliness may be similar, the social, cognitive, and neural sequelae may shift across the adult lifespan. In the present study, we directly examine how individual and age differences in sociality–loneliness and empathic responding– relate to the intrinsic network architecture of the brain. We focus our analysis specifically on brain regions within six networks previously implicated in loneliness in younger and older adults (Lam et al., 2021; Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020): visual, dorsal attention, ventral attention, limbic, frontoparietal, and default networks. We test the prediction that diverging loneliness-related RSFC patterns previously identified in young and middle-aged adults will be observed when directly comparing younger and older adults. Specifically, we hypothesize that younger adults will show a consistent pattern as Mwilambwe-Tshilobo et al. (2019) characterized by greater functional integration of the default network with visual and attention networks. In contrast, older adults will show greater functional integration of the default network with frontoparietal and limbic networks that more closely aligns with the patterns observed in middle-aged adults (Spreng et al., 2020). Consistent with research indicating that older adults prioritize close social relationships (Carstensen, 1992) and that loneliness alters brain regions implicated in social functioning (J. Cacioppo & Hawkley, 2009; Kanai et al., 2012), we hypothesized that the RSFC patterns positively associated with loneliness would be inversely related to empathic responding and more robustly expressed in older adults than younger adults.

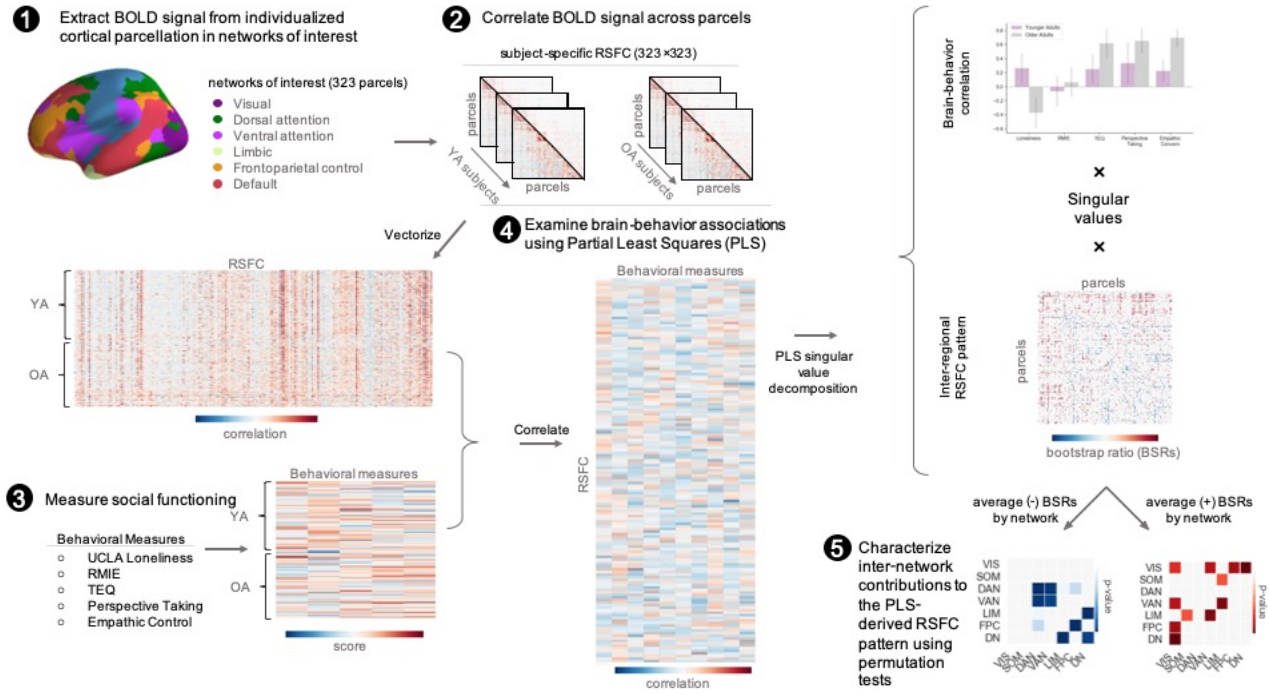


Figure 4.1: Caption. Analytic workflow of individual and age differences in functional connectivity related to loneliness and empathic responding. (1) BOLD resting-state data were extracted from subject-specific individual parcellation in six networks of interest: visual, dorsal attention, ventral attention, limbic, frontoparietal control, and default networks. (2) Functional connectivity between parcels were constructed, forming a 323×323 matrix. The lower triangle of each subject's matrix was vectorized and arranged by group assignment into a larger RSFC matrix. (3) Younger and older adults' scores on behavioral measures of loneliness and empathic responding were combined into a matrix. (4) Partial Least Squares (PLS) was used to identify patterns of RSFC that maximally covary with the behavioral measures across subjects. A cross-correlation matrix generated by multiplying the RSFC and behavioral matrix was submitted to singular value decomposition. (5) Network contribution plots were used as a metric of the most reliable intra- and inter-network connections by summarizing the inter-regional connections from the PLS matrix.

4.5 Methods

4.5.1 Participants

Data from 220 participants were analyzed in the present study. Participants were part of a larger cohort (Setton et al., 2022), where inclusion required both loneliness and empathy assessments, and two resting state fMRI runs of data. Participants in the final sample included 128 younger (Mage = 22.6 years, SD = 3.3; range = 18-34; 75% female) and 92 older (Mage = 69 years, SD = 6.6, range = 60-89; 47% female) healthy adults recruited in Ithaca, New York and Toronto, Canada (Table). All participants were right handed ranging from 18 to 89 years (M= 42 SD = 23.5). All participants provided informed consent in accordance with the guidelines set by the Institutional Review Board at Cornell University and York University.

4.5.2 Behavioral Measures

Loneliness measures

Loneliness was measured using the Revised UCLA Loneliness Scale (UCLA-LS Russell, 1996). The UCLA-LS is a 20-item questionnaire that measures subjective feelings of loneliness and perceived social isolation (Russell, 1996). This measure is well established within the literature and found to be highly reliable (Russell, 1996). One of the advantages of the UCLA-LS questionnaire is that it assesses loneliness indirectly, which diminishes potential response bias (Shiovitz-Ezra & Ayalon, 2012). For example, participants are asked to respond to statements such as 'How often do you feel like there is no one you can turn to?' or 'How often do you feel isolated from others'. Responses were provided on a 4-point Likert scale ranging from 1 (Never) to 3 (Always). Negatively-worded items were scored in reverse. Higher scores reflect higher self-reported loneliness.

Aspects of empathic responding

Empathy is not a unitary concept, but rather a multidimensional construct that can be broken down into cognitive and affective components. The cognitive components of empathy describe processes that underlie the ability to understand and make inferences regarding another person's mental states. The affective components of empathy describe the emotional reaction towards the observed experiences of another. While distinctions regarding these two behavioral processes are made within the literature, recent work suggests that overlapping and unique brain activation patterns support the ability to understand how other people think and feel (Schurz et al., 2021). Therefore, to ensure that our assessment of empathic responding reflected these cognitive and affective neurocognitive processes, participants completed a performance-based assessment of emotional recognition and two self-report questionnaires that represent subdomains of empathy along these two dimensions:

1. The Reading the Mind in the Eyes (RMIE) task was originally conceptualized as a theory of mind questionnaire (Baron-Cohen et al., 2001), however it has been shown to actually measure emotional recognition and not theory of mind (Oakly et al., 2016). We included the RMIE as a task-based measure in our analysis because emotional recognition is critical aspect of empathic responding that is also predictive of prosociality (Bailey et al., 2020). The RMIE task consists of 36 photos of the eye region of adults expressing different emotional states. Participants were asked to choose one adjective from a list of four that best expresses the internal state depicted in the photo. One point was assigned for each correct response, 0 points for incorrect responses, and negatively-worded items scored in reverse. Individual items were summed to give a total score of 36 with higher scores indicating higher emotional decoding.

2. The Toronto empathy questionnaire (TEQ) is a self-report measure that primarily assessed emotional empathy (Spreng et al., 2009). It consists of 16 items in which

participants respond on a 5-point Likert scale ranging from 0 (Never) to 4 (Always). Negatively-worded items were scored in reverse. Examples of items in the TEQ include "I can tell when others are sad even when they do not say anything" and "When I see someone being treated unfairly, I do not feel very much pity for them."

3. The Interpersonal Reactivity Index (Davis, 1980)is a self-report questionnaire that consists of 4 subscales that assess different aspects of empathy: (1) Perspective taking (PT), the ability to take another person's psychological point of view; (2) Fantasy, the ability to project one-self onto fictional characters; (3) Empathic concern (EC)¬¬, the tendency to experience feelings of sympathy and compassion for others; and (4) Personal distress, a measure of the aversive response one feels when observing the negative experience of others. For this study, we only included measures of PT and EC because we were specifically interested in assessing cognitive and affective aspects of empathic responding that were other-focused (i.e. PT, EC) rather than self-focused (Fantasy, Personal Distress). Each subscale had 7-items with responses made on a 5-point Likert scale ranging from 1= "Does not describe me well" and 5= "Describes me well". Negatively-worded items were scored in reverse.

Covariates

Several demographic, social, cognitive, and personality variables associated with loneliness and aging were included as covariates in our analyses. Demographic variables included age, gender, and educational attainment. We also included the study site as a covariate since participants were part of a multi-site cohort study (Spreng et al., 2022). Since our study focused on identifying age-related differences in the behavioral and neural associations between loneliness and empathy, we needed to account for known age-dependent factors that influence social and brain functioning. Age differences in loneliness are due to differences in the distribution of risk factors. For example, older adults accumulate disproportionate risk factors contributing to loneliness (Luhmann & Hawkley, 2016), two of which are poor cognitive functioning and social isolation. Evidence suggests

that loneliness may accelerate cognitive decline among older adults (Shankar et al., 2013; Wong et al., 2016), and previous work highlights the need to account for the confounding effects of objective social isolation when examining loneliness among older adults (Steptoe et al., 2013). Age differences in loneliness may also be due to normative age-related changes in the quantity and quality of social relationships. Aging is marked by significant transitions in the size and composition of social relationships that lead to shrinking social network size to prioritize close social ties (L. Carstensen, 1992). Social relationship quantity and quality are negatively correlated with loneliness. However, having few high-quality relationships is a much stronger predictor of loneliness (Luhmann & Hawkley, 2016). Thus, accounting for the quantity and quality of social relationships may be important factors in how both age groups experience loneliness. Therefore, we controlled for objective social isolation, relationship quality, and global cognitive function measures to account for normative social network size and cognitive declines. We used the Social Network Index (Cohen et al., 1997), NIH Toolbox Emotion Battery, and Cognition Battery (http://www.nihtoolbox.org]), respectively:

1. The Social Network Index is a self-report questionnaire that assesses various aspects of social engagement with 12 different types of social relationships (e.g., spouse, children, relative, friend, neighbor, co-worker). Participants were asked to indicate the number of people they regularly talk to or see at least once every two weeks for each relationship type. The total number of people identified was summed to estimate social network size.

2. The NIH Toolbox Emotion Battery included three measures where participants were asked to report on their perception of social support and friendship available to them by others in their social networks (Salsman et al., 2013): (1) instrumental support: the subjective perception that others in their social network are available to provide advice in times of need; (2) emotional support: the subjective perception that people in their social network are available to listen to one's concerns with un-

derstanding and caring; and (3) friendship: the subjective perception that they have companions/ friends available to them with which they can interact.

3. The NIH Toolbox Cognition Battery included a global composite score of overall cognition, which was automatically computed by averaging scores across seven cognitive function tests: the Picture Vocabulary Test and Oral Reading Recognition Test, Dimensional Change Card Sort Test, the Flanker Inhibitory Control and Attention Test, the Picture Sequence Memory Test, the List Sorting Working Memory Test, and the Pattern Comparison Processing Speed Test (Gershon et al., 2013). Higher scores represent better performance.

Beyond social isolation and cognition, certain personality traits may be risk factors for loneliness. Neuroticism is a personality trait that strongly positively correlates with loneliness (Abdellaoui et al., 2019). In addition, neuroticism has been associated with cognitive decline (D'Iorio et al., 2018). It mediates the relationship between loneliness and structural changes to dorsolateral prefrontal cortex (X. Kong et al., 2015). To account for the potential contribution of neuroticism when examining age differences in our analyses, we included neuroticism as a covariate. Participants completed The Big Five Aspect Scale (DeYoung et al., 2007), which is a 100-item self-report questionnaire that assesses facets of personality traits.

4.5.3 Behavioral Data Analysis

We first conducted an independent samples t-test to compare younger and older adults on all behavioral measures, including covariates. This allowed us to determine whether there were any age-related differences in self-reported loneliness and empathy. We then performed product-moment and partial correlations analyses to characterize the associations among all behavioral measures. Next, we examined associations between loneliness and each measure of empathic responding (RMIE, TEQ, IRI perspective taking, IRI empathic concern) in the full sample and separately within each age group. All covari-

ates were included in partial correlation models (age, gender, site, education, neuroticism, cognitive composite score). The partial correlation analysis excluded participants with missing data on any of the covariate measures. In addition, given that a sizable portion of participants had missing data on the social network size measure (young adults: n = 32; older adults: n = 20), additional partial correlation analyses that included social network size as a covariate were conducted only in participants with complete behavioral data. Subjective measures of instrumental support, emotional support, and friendship were also included. Statistical significance was set at p

4.5.4 Neuroimaging

Imaging data were acquired on a 3T GE750 Discovery series MRI scanner with a 32-channel head coil at the Cornell Magnetic Resonance Imaging Facility in Ithaca, NY or on a 3T Siemens Tim Trio MRI scanner with a 32-channel head coil at the York University Neuroimaging Center in Toronto, Canada. Scanning protocols were closely matched across sites. Anatomical scans at Cornell were acquired using a T1-weighted volumetric magnetization prepared rapid gradient echo sequence (TR=2530ms; TE=3.4ms; 7° flip angle; 1mm isotropic voxels, 176 slices, 5m25s) with 2x acceleration with sensitivity encoding. At York, anatomical scans were acquired using a T1-weighted volumetric magnetization prepared rapid gradient echo sequence (TR=1900ms; TE=2.52ms; 9° flip angle; 1mm isotropic voxels, 192 slices, 4m26s) with 2x acceleration and generalized auto calibrating partially parallel acquisition (GRAPPA) encoding at an iPAT acceleration factor of 2. Two 10m06s resting-state runs were acquired using a multi-echo (ME) EPI sequence at Cornell University (TR=3000ms; TE1=13.7ms, TE2=30ms, TE3=47ms; 83° flip angle; matrix size=72x72; field of view (FOV)=210mm; 46 axial slices; 3mm isotropic voxels; 204 volumes, 2.5x acceleration with sensitivity encoding) and York University (TR=3000ms; TE1=14ms, TE2=29.96ms, TE3=45.92ms; 83° flip angle; matrix size=64x64; FOV=216mm; 43 axial slices; 3.4x3.4x3mm voxels; 200 volumes, 3x acceleration and GRAPPA encoding).

Participants were instructed to stay awake and lie still with their eyes open, breathing and blinking normally in the darkened scanner bay.

Processing

Anatomical images were skull stripped using the default parameters in FSL BET (Smith, 2002). Brain-extracted anatomical and functional images were submitted to ME independent component analysis (ME-ICA; version 3.2 beta; https://github.com/ME-ICA/me-ica; Kundu et al., 2011; Kundu et al., 2013). ME-ICA relies on the TE-dependence model of the BOLD signal to determine T2* in every voxel and separates the BOLD signal from non-BOLD sources of noise. Before TE-dependent denoising, time series data were minimally preprocessed: the first 4 volumes were discarded, images were computed for de-obliquing, motion correction, and anatomical-functional coregistration, and volumes were brought into spatial alignment across TEs. The T2* maps were then used for anatomical-functional coregistration. Grey matter and cerebrospinal fluid compartments are more precisely delineated by the T2* map than by raw EPI images (Kundu et al., 2017; Speck et al., 2001), which is an important consideration in aging research where enlarged ventricles and greater subarachnoid space often blur these boundaries.. Volumes were then optimally combined across TEs and denoised. The outputs of interest included: 1) spatial maps consisting of the BOLD components, 2) reconstructed time series containing only BOLD components, and 3) the BOLD component coefficient sets.

ME-ICA effectively removes distant-dependent motion-related artifacts in the fMRI data (J. D. Power et al., 2018). To retain all trials and maintain the same time series length across participants, we did not implement any additional denoising steps, such as scrubbing. Instead, we perform an image quality assessment on the denoised time series. In native space, we identified and excluded participants with unsuccessful coregistration, residual noise (framewise displacement (FD) ¿ .50 mm coupled with denoised time series showing DVARS ¿1; (J. Power et al., 2012)), temporal signal to noise ratio ¡ 50, or fewer than ten retained BOLD-like components. Forty participants were excluded after the im-

age quality assessment (younger adults: n= 12; older adults: n = 28). Age group and site differences in residual motion for included participants were assessed using FD calculated on the middle echo prior to processing. Statistical results are reported in Supplementary Table .

The denoised BOLD component coefficient sets in native space, optimized for RSFC analyses (Kundu et al., 2013), were used in subsequent steps. We refer to these as multi-echo functional connectivity (MEFC) data. Additional measures were taken to account for variation in the number of independent components from ME-ICA once connectivity matrices were estimated, as detailed below. MEFC neuroimages were mapped to a common cortical surface for each participant using FreeSurfer v6.0.1 (Fischl, 2012). To maximize alignment between intensity gradients of structural and functional data (Greve & Fischl, 2009), MEFC data were first linearly registered to the T1-weighted image by run. The inverse of this registration was used to project the T1-weighted image to native space and resample the MEFC data onto a cortical surface (fsaverage5) with trilinear volume-to-surface interpolation. This produces a cortical surface map where each vertex, or surface point, is interpolated from the voxel data. Once on the surface, runs were concatenated and MEFC data at each vertex were normalized to zero mean and unit variance.

Individualized RSFC parcellation

We generated participant-specific functional connectomes to examine individual differences in functional brain network organization using the Group Prior Individual Parcellation (GPIP; Chong et al., 2017). This approach enables a more accurate estimation of participant-specific individual functional areas (Chong et al., 2017) and is more sensitive to detecting RSFC associations with behavior (e.g., (R. Kong et al., 2021; Setton et al., 2022). The main advantage of this approach is that the correspondence among parcel labels is preserved across participants, while the parcel boundaries are allowed to shift based on the individual-specific functional network organization of each participant—thus providing a similar RSFC pattern that is shared across the population.

Starting from an initial pre-defined group parcellation atlas, GPIP first refines each individual's parcel boundaries relative to their resting-state fMRI data. Next, the concentration (inverse covariance/partial correlation) matrices from all subjects are jointly estimated using a group sparsity constraint. GPIP iterates between these two steps to continuously update the parcel labels until convergence, defined as no more than one vertex changing per parcel or 40 iterations. Compared to other group-based parcellation approaches, GPIP has been shown to improve the homogeneity of the BOLD signal within parcels and the delineation between regions of functional specialization (Chong et al., 2017).

Using this method, we used the MEFC data from each participant and parcellated the cortex into 400 functionally defined regions. We initialized all participants to a group parcellation atlas developed by Schaefer et al. (2018). Each parcel was matched to a corresponding network in the 7 network parcellation by Yeo et al. (2011). In the present report, we included the visual, dorsal attention, ventral attention, limbic, frontoparietal control, and default networks given their reliable associations with loneliness across the neuroimaging literature (Lam et al., 2021). In addition, as described in the introduction these networks have been associated with loneliness in younger (Mwilambwe-Tshilobo et al., 2019) and older adults (Spreng et al., 2020). While we could have excluded the somatomotor network, recent evidence suggests robust age differences in RSFC (Setton et al., 2022), which were orthogonal to the predictions tested here. For completeness, the results for the full seven network analysis are reported in supplemental material (Supplementary Figure 4.S1-4.S2).

Partial Least Squares (PLS) analysis

PLS is a data-driven multivariate statistical technique used to decompose relationships between two datasets (functional connections and behavioral measures) into orthogonal sets of latent variables that maximally covary together across participants (McIntosh & Lobaugh, 2004). The latent variables can be interpreted as optimally-paired functional

networks and behavioral phenotypes, respectively. We used PLS to identify age-related differences and similarities in RSFC that were directly correlated to loneliness and empathy.

Two datasets were constructed: a **Y** matrix containing participant's behavioral scores on loneliness and empathy measures, and a **X** matrix consisting of participants' functional connectomes. Each row of the **Y** and **X** matrices represents the number of participants organized by group. The columns of matrix **X** correspond to the edges of the vectorized lower triangle of the RSFC matrix. The **X** and **Y** matrices were mean centered, normalized, and a correlation matrix (**R = X'Y**) was submitted to singular value decomposition (SVD) as follows:

$$\mathbf{R = X'Y = USV'}$$

SVD of the cross-correlation matrix **X'Y** produced multiple mutually-orthogonal latent variables, each consisting of three elements:

1. A left singular vector (**U**) containing weights for each of the behavioral measures.
2. A right singular vector (**V**), containing weights for each of the functional connections that best characterize the relationship between RSFC among younger and older adults.
3. A scalar singular value (**S**).

Squared singular values reflect effect sizes which are proportional to the covariance between RSFC and behavior that is accounted for by each latent variable. The number of latent variables is sorted in order of proportion of covariance between the RSFC and behavior measures.

Participant-specific brain scores

For each latent variable, we derived participant-specific brain scores that assess the extent to which each participant contributes to the group covariance RSFC pattern. The brain scores were calculated by multiplying the original matrix of participants' functional

connectomes (**X**) with the PLS-derived right singular vector (**V**). To account for possible confounds in the brain-behavior correlation, partial correlations between the brain scores and each behavioral measure was conducted controlling for covariates of no interest (age, gender, site, education, neuroticism, and cognitive composite score). Covariates were partialled-out of both the brain scores and behavioral measures.

Permutation tests

The significance of each latent variable was assessed using permutation testing. Rows of **X** were randomly reordered and subjected to SVD iteratively, as described above. This was done 10,000 times, creating a distribution of singular values under the null distribution (McIntosh & Mišić, 2013). A p-value was computed for each latent variable as the proportion of permuted singular values greater than or equal to the original singular value. Critically, permutation tests involve the entire multivariate pattern and are performed in a single analytic step, so correction for multiple comparisons is not required (McIntosh & Lobaugh, 2004).

Bootstrap resampling

The reliability of the weights of individual RSFC connections and behavior were assessed using bootstrap resampling (Krishnan et al., 2011; McIntosh & Mišić, 2013). The brain-behavior correlations were calculated using 10,000 bootstrap samples. To identify individual connections that made a statistically significant contribution to the overall RSFC pattern, we calculated the ratio between each weight in the singular vector and its bootstrap-estimated standard error. Bootstrap ratios are equivalent to z-scores if the bootstrap distribution is approximately unit normal (Efron & Tibshirani, 1986). Bootstrap ratios were therefore thresholded at values of ± 1.96, corresponding to the 95% CI.

Cross-validation

To assess the reliability of our PLS analysis, we conducted a train-test validation of the PLS results using 5-fold cross-validation (Kebets et al., 2019). We assigned 80% of the participant data in each age group to a train set and the remaining 20% to a test set. For each fold, we used PLS to compute the RSFC (**Utrain**) and behavioral (**Vtrain**) singular vectors. Then we projected the test data onto the singular vectors from the training data, allowing us to estimate participant-specific brain scores and correlation for the test set (corr(**Xtest Utrain** , **Ytest Vtrain**)). This was done over 5 folds, and the correlations between the test set original **X** (RSFC) and **Y**(behavior) matrix was performed for LV1 and LV2. The significance of the correlation was assessed using permutation tests (1000 repetitions on the behavioral data within each group).

Supplementary control analyses

We performed three additional analyses to account for possible confounding effects of the quantity and quality of social relationships, age, and motion on the primary PLS findings. First, confirmed that age group differences in the relationship between loneliness and RSFC were not due to differences in either the quantity or quality of relationships among younger and older adult participants. Therefore, two separate partial correlation analyses were including using social network size (quantity) and subjective measures of social support and friendship (quality). Brain-behavior correlations for the primary PLS results were computed and reported in Supplementary Table 4.S3 and 4.S4, respectively.

The second control analysis was performed to confirm that participants' age did not influence the age differences captured in the primary PLS analysis. Age was used as a continuous variable and partialled out from the original **X** and **Y** input matrices. The two matrices were then used to run a new PLS analysis (see Supplementary Figure 4.S5 - 4.S6 and Supplementary results). Next, we compared the covariance of each LV before and after partialling out age (Supplementary Figure 4.S7) to evaluate whether partialling out

age decreased the effect size, which would be indicative of the confounding influence of age in our findings.

The last control analyses examined residual motion's impact on RSFC in our sample. First, two independent PLS analyses were performed: (1) examining the association between RSFC and mean FD (pre-processing) and (2) identifying age differences in whole-brain RSFC (no behavior). To confirm that the RSFC pattern covarying with FD was not associated with age differences in RSFC, we correlated the brain scores derived from each PLS analysis. Relationships are plotted for the entire sample and separately for younger and older adults (Supplementary Figure 4.S8). Finally, to account for the effects of motion on the primary PLS analysis, mean FD post-processing was included as an additional covariate (Supplementary Table 4.S6). Results are reported in the supplementary section.

Network Contribution Analysis

In addition to assessing the contribution of inter-regional connections to the group differences, we also evaluated the extent to which network-level RSFC within and between functional networks contributed to group differences. Two separate weighted adjacency matrices were constructed from positive and negative RSFC weights by quantifying the network-level contributions to the PLS-derived RSFC pattern. For both matrices, nodes represent parcels defined by the individual parcellation, while edges correspond to the thresholded bootstrap ratio of each pairwise connection. Network-level RSFC contributions were estimated by assigning each parcel of the Schaefer atlas according to their respective network label based on the assignment reported by Yeo et al. (2011) and taking the average of all connection weights in a given network, thereby generating a 6 x 6 matrix. During each permutation, network labels for each node were randomly reordered and the mean intra- and inter- network RSFC were recalculated. This process was repeated 1000 times to generate an empirical null sampling distribution that indicates no relationship between network assignment and RSFC pattern (Mirchi et al., 2019). The mean contribution for all intra- and inter-network network connections expressed as z-

scores relative to the permuted null model are shown in Supplementary Figure 4.S4. The significance of the pairwise connections to the network matrix was determined by estimating the proportion of times the value of the sampling distribution was greater than or equal to the original value.

4.6 Results

We measured self-reported loneliness and used self-report measures and task performance to assess cognitive and emotional aspects of empathic responding (See Table 4.1). We hypothesized that loneliness would be inversely related to empathic responding across the lifespan (Beadle et al., 2012; Nakagawa et al., 2015). Further, we predicted that these associations might be more robust in later life as the detection of social cues declines (Denburg & Hedgcock, 2015; Moran et al., 2012) and socioemotional goals become increasingly salient (L. L. Carstensen et al., 1999). Next, we examined age-related differences in the association between loneliness, aspects of empathic responding, and cortical RSFC. We acquired twenty minutes of multi-echo resting-state fMRI data (Kundu et al., 2017) and applied individualized parcellation to a subset of individuals previously examined to assess age differences in the functional architecture of the brain (Setton et al., 2022). Multivariate PLS (McIntosh & Mišić, 2013; Schurz et al., 2021; Spreng et al., 2020) was used to identify patterns of RSFC related to individual differences in loneliness and empathic responding, as well as differences between younger and older adult age groups. Based on our previous findings of distinct young and middle-aged adult patterns (Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020), we predicted robust age differences in the association between loneliness, aspects of empathic responding, and RSFC. Specifically, we hypothesized that age differences would arise within the default network and default network interactions with other association networks implicated in internally-directed cognitive processes (Andrews-Hanna et al., 2014).

4.6.1 Demographics and descriptive statistics

To examine whether the relationship between loneliness and empathic responding showed similar associations as prior studies (Beadle et al., 2012; Nakagawa et al., 2015), we first characterized individual and age-related differences in loneliness and subdomains of empathy within our cohort. The behavioral measures included self-reported loneliness, emotional recognition accuracy on the RMIE task, self-reported empathy, perspective taking and empathic concern. Additionally, we controlled for nuisance or confounding variables, including scanning site, gender, education, social network size, instrumental support, emotional support, friendship, neuroticism, and global cognition (see Methods for full rationale). Table 4.1 summarizes the means and standard deviations of loneliness and empathic responding measures, along with all covariates included in subsequent analyses.

Table 4.1: Descriptive data (mean and standard deviations) and inferential statistics for behavioral measures in younger and older adults.

		Overall	Younger Adults	Older Adults	Significance
Demographics					
n		220	128	92	
Age, mean (SD)		42.0 (23.5)	22.6 (3.3)	69.0 (6.6)	
Gender, n (%)	F	122 (55.5)	75 (58.6)	47 (51.1)	
	M	98 (44.5)	53 (41.4)	45 (48.9)	
Education, mean (SD)		16.1 (2.6)	15.2 (1.8)	17.5 (2.9)	<0.001***
Social Measures					
UCLA Loneliness Scale, mean (SD)		39.6 (9.1)	40.6 (9.4)	38.2 (8.5)	0.06
SNI Size, mean (SD)		22.5 (12.5)	23.7 (12.4)	20.9 (12.4)	0.16
Instrumental Support, mean (SD)		31.1(7.6)	31.7(8.7)	30.6(6.8)	0.3
Emotional Support, mean (SD)		33.9(5.4)	32.8(5.4)	34.7(5.4)	0.02*
Friendship, mean (SD)		31.3(6.3)	29.9(6.6)	32.3(5.9)	0.01**
Empathic Functioning					
Reading the Mind in the Eyes (RMIE), mean (SD)		72.2 (10.0)	74.4 (9.8)	69.2 (9.7)	<0.001***
Toronto Empathy Questionnaire (TEQ), mean (SD)		39.0 (3.9)	39.1 (4.1)	38.8 (3.6)	0.65
IRI Perspective Taking (PT), mean (SD)		2.8 (0.6)	2.7 (0.6)	2.8 (0.6)	0.18
IRI Empathic Concern (EC), mean (SD)		3.0 (0.5)	2.9 (0.5)	3.1 (0.5)	0.003**
Personality					
Neuroticism, mean (SD)		2.5 (0.7)	2.7 (0.7)	2.2 (0.6)	<0.001***
Cognition					
NIH Cognitive Composite Score, mean (SD)		126.6 (14.4)	131.4 (14.7)	119.9 (10.9)	<0.001***

Note. IRI = Interpersonal Reactivity Index, SD = standard deviation. * p< 0.05; ** p< 0.01. *** p< 0.001

Violin plots illustrating age differences on behavioral measures in each age group are shown in Figure 4.2. Scores on the UCLA loneliness scale trended lower for older, compared to younger adults (t(218) = 1.88, p = 0.06; Cohen's d=0.26; Figure 4.2A). Younger and older adults significantly differed on some, but not all, measures of empathy. Older adults were less accurate at emotional recognition of others' facial expressions based on performance on the RMIE (t(218) = 3.94, p $<$ 0.0001; Cohen's d=0.54; Figure 4.2F). Older adults reported greater empathic concern than younger adults (t(218) = -3.00, p = 0.003; Cohen's d=0.41; Figure 4.2I). No significant age differences were found on other measures of empathic functioning (TEQ: t(218) = 0.45, p = 0.65; Cohen's d=0.06; Perspective Taking: t(218) = -1.35, p = 0.18; Cohen's d=0.19; Figure 4.2G-H).

Neuroticism and normative cognition declines may influence the relationship between loneliness, empathic responding, and RSFC. Therefore, we assessed age-related differences in neuroticism and global cognition based on the NIH cognitive composite score.We also assessed whether younger and older adults differed across covariates incorporated

in subsequent analyses. Older adults scored lower on neuroticism (t(207.79) = 5.32, p $<$ 0.001; Cohen's d=0.72; Figure 4.2K) and had lower overall cognitive function (t(210) = 6.2, p $<$ 0.001; Cohen's d=0.86; Figure 4.2J). Social networks tend to shrink with age, and evidence from longitudinal work has found that objective social isolation may confound the effects of loneliness among older adults (Steptoe et al., 2013). To determine whether such differences were present within our sample, we compared the network sizes between younger and older adults and found no age difference (t(166) = 1.42, p = 0.16; Cohen's d=0.22; Figure 4.2B). We also included measures of perceived social support and friendship to assess the quality of social relationships participants felt they had access to. Older adults reported greater perceived emotional support (t(215) = 2.45, p = 0.02; Cohen's d=0.34; Figure 4.2D) and friendship (t(215) = 2.83, p = 0.01; Cohen's d=0.39; Figure 4.2E), but no significant differences were found for instrumental support (t(215) = -1.08, p = 0.28; Cohen's d=0.15; Figure 4.2C).

Next, we assessed the association among all behavioral variables across the full sample (see Table 4.6.1). Scores on the UCLA loneliness scale correlated negatively with perspective taking, empathic concern, social network size, instrumental support, emotional support, friendship. Scores on the UCLA loneliness scale were positively associated with neuroticism and cognitive function. Accuracy on the RMIE was not significantly associated with loneliness or other empathy subdomain measures. RMIE accuracy in this sample was positively correlated with emotional support and cognitive function. The TEQ was correlated with perspective taking and empathic concern subscales of the IRI as well as with participant social network size. Empathic concern was positively correlated with perspective taking, emotional support, and friendship, and negatively associated with neuroticism and cognitive function. Social network size was positively correlated with friendship. Emotional support was positively with friendship. Neuroticism was negatively correlated with emotional support and friendship, and contrary to expectation, positively correlated with global cognitive function.

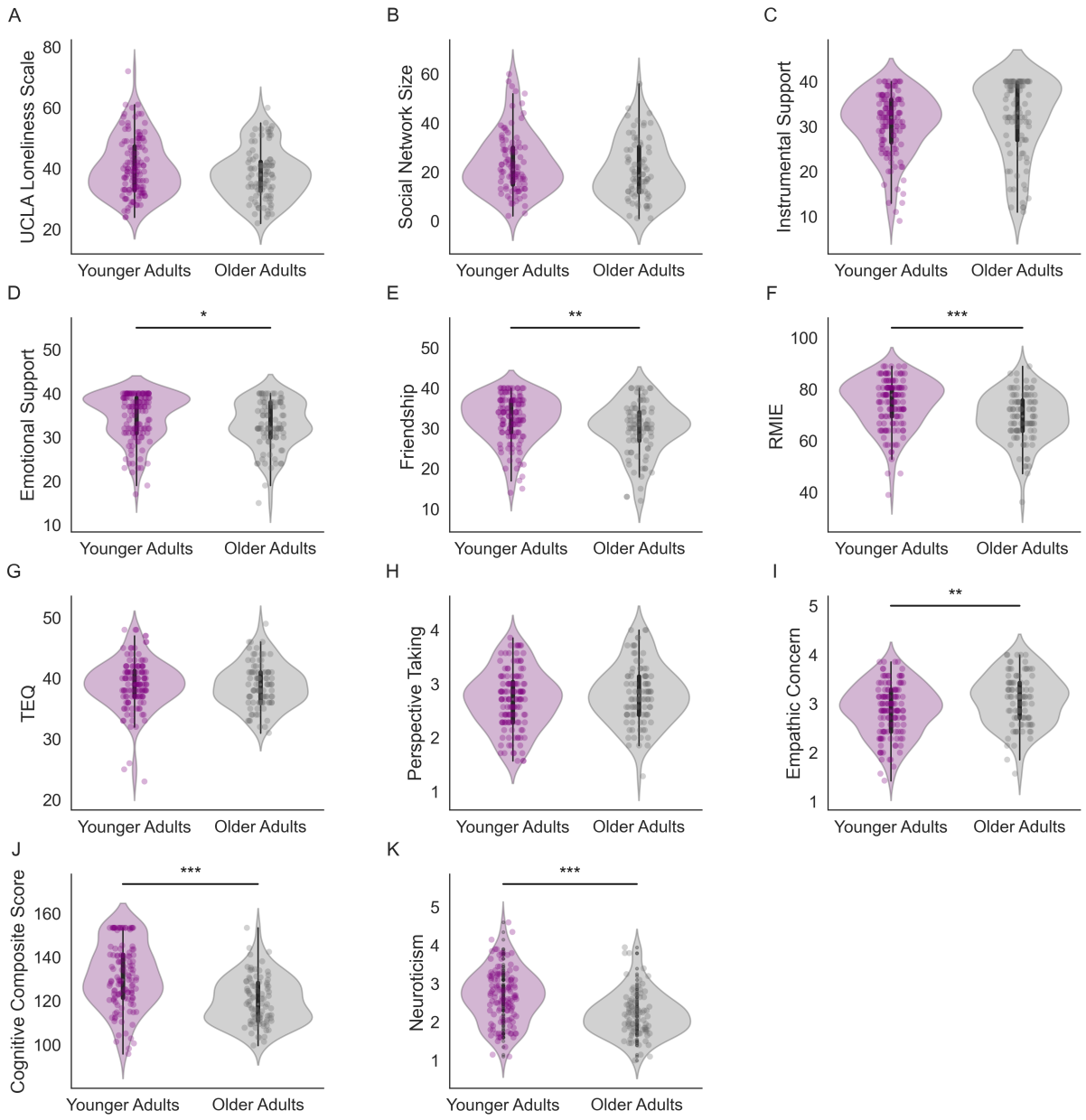


Table 4.2: Caption. Group comparison on behavioral measures. Violin plots showing the distribution of behavioral scores in younger and older adults for (A) loneliness, (B) social network size, (C-D) social support, (E) friendship, (F-I) empathic responding measures, (J) global cognitive function, (K) and neuroticism. For comparisons on behavioral measures of interest (loneliness and empathic responding), although self-reported loneliness was similar among age groups, significant age-related differences can be observed for task-based performance of emotional recognition and in self-reported empathic concern. RMIE= Reading the Mind in the Eyes Task; TEQ= Toronto Empathy Questionnaire. ** indicates $p < 0.01$. *** indicates $p < 0.001$.

Table 4.2: Descriptive data (mean and standard deviations) and inferential statistics for behavioral measures in younger and older adults.

	1	2	3	4	5	6	7	8	9	10	11	12
1. Loneliness	-											
2. RMIE	0.07	-										
3. TEQ	-0.12	0.14	-									
4. IRI Perspective Taking	**-0.16***	0.06	**0.23****	-								
5. IRI Empathic Concern	**-0.20***	0	**0.45*****	**0.42*****	-							
6. Social Network Size	**-0.21****	0.02	**0.24****	0.1	**0.20***	-						
7. Instrumental Support	**-0.31*****	-0.02	0.02	0.04	0.03	-0.02	-					
8. Emotional Support	**-0.56*****	**0.21****	0.11	**0.16***	0.06	0.01	**0.40*****	-				
9. Friendship	**-0.68*****	0.06	0.11	**0.20***	0.08	**0.26****	**0.25****	**0.59*****	-			
10. Neuroticism	**0.46*****	0.11	0.08	**-0.34*****	**-0.19***	0.01	-0.12	**-0.31*****	**-0.27*****	-		
11. Education	-0.1	-0.04	-0.06	-0.03	0.14	-0.01	0.13	-0.07	-0.12	-0.06	-	
12. Cognition Composite Score	**0.18***	**0.40*****	0.07	-0.04	-0.15	0.06	-0.15	-0.14	-0.05	**0.29*****	-0.01	0.18

Note: RMIE= Reading the Mind in the Eyes Task; TEQ= Toronto Empathy Questionnaire. Social Network Size, Neuroticism, Education, and Cognition Composite Score are included as covariates in analyses.

* indicates $p < 0.05$. ** indicates $p < 0.01$. *** indicates $p < 0.001$.

4.6.2 Behavioral associations between loneliness and empathic responding in younger and older adults

Our previous results showed significant age differences on some aspects of empathic responding. We therefore examined the association between loneliness and empathy measures in both younger and older adults separately (controlling for gender, site, education, neuroticism, and global cognitive function). The partial correlations between the gold-standard UCLA loneliness scale and the four measures of empathic responding are shown in Supplementary Table 4.1 for both age groups. In younger adults, loneliness was not significantly correlated with any empathic responding measures. In contrast, loneliness in older adults was significantly and negatively associated with TEQ, perspective taking and empathic concern, but not accuracy on the RMIE task. Two additional partial correlation correlations were performed to assess the influence of social network quantity and quality on the behavioral associations observed in younger and older adult (Supplementary Table 4.S1B. Although not all participants in the cohort completed the objective social network size measure, we reanalyzed the associations between loneliness and empathic responding by including social network size as a covariate and found that no significant associations remained (Supplementary Table 4.S1C). However, when we included instrumental support, emotional support, and friendship as proxy measures of social relationship quality loneliness across the full sample was significantly correlated with accuracy on the RMIE while all remaining measures of empathic functioning were no longer significant (Supplementary Table 4.S1D).

4.6.3 RSFC associations with loneliness and empathic responding in younger and older adults

Next, we implemented a data-driven multivariate approach to identify patterns of RSFC related to loneliness and empathic responding in younger and older adults (Figure 4.1. RSFC was examined among the visual, dorsal attention, ventral attention, limbic, fron-

toparietal, and default networks. Two significant LVs capturing distinct RSFC patterns reflecting age-related differences and similarities in social behavior were observed. A scree plot showing the covariance explained for all LVs is shown in Supplementary Figure 4.S3. Detailed results examining the impact of social relationship quantity and quality on brain-behavior associations identified by each LV are provided in Supplementary Results 4.S1.

Age differences in RSFC related to loneliness

The first LV revealed a pattern of RSFC that dissociated younger and older adult RSFC associated with loneliness (p = 0.04; 26.02% covariance explained; Figure 4.3). Additionally, self-reported empathy covaried in both groups with a pattern of RSFC observed for loneliness in young adults. No reliable relationship between emotional recognition on the RMIE task and RSFC was found in either age group. To assess the specificity of the brain-behavior correlations in each group, we performed a partial correlation analysis controlling for the effects of gender, site, social network size, neuroticism, and cognitive function on participants' brain scores. Figure 4.3B-F depicts scatterplots of the relationship between participant brains scores, representing the weighted values of the RSFC pattern of the LV controlling for covariates and all five behavioral measures. Results indicate that significant brain-behavior correlations for LV1 were robust, as they remained significant after controlling for covariates in both age groups (see Supplementary Table 4.S2 for statistical results).

The contribution of each network to the pattern of RSFC of LV1 is shown in Figure 4.3G for positive (Figure 4.3H) and negative (Figure 4.3I) associations with RSFC. The most notable feature that emerged was a dissociation between connectivity of the visual network and heteromodal association regions. This dissociation reflects the age interaction in loneliness on RSFC. In younger adults, higher loneliness was associated with greater connectivity of the visual network with the ventral attention, frontoparietal control, and default networks, as well as greater limbic to ventral attention connectivity (Figure 4.3H).

This pattern of RSFC was also associated with self-reported empathy (TEQ, perspective taking, and empathy) in both age groups. In contrast, higher loneliness in older adults was associated with more intra-network RSFC of attention, limbic, frontoparietal, and default networks, as well as greater RSFC between dorsal and ventral attention, and default and limbic networks (Figure 4.3I).

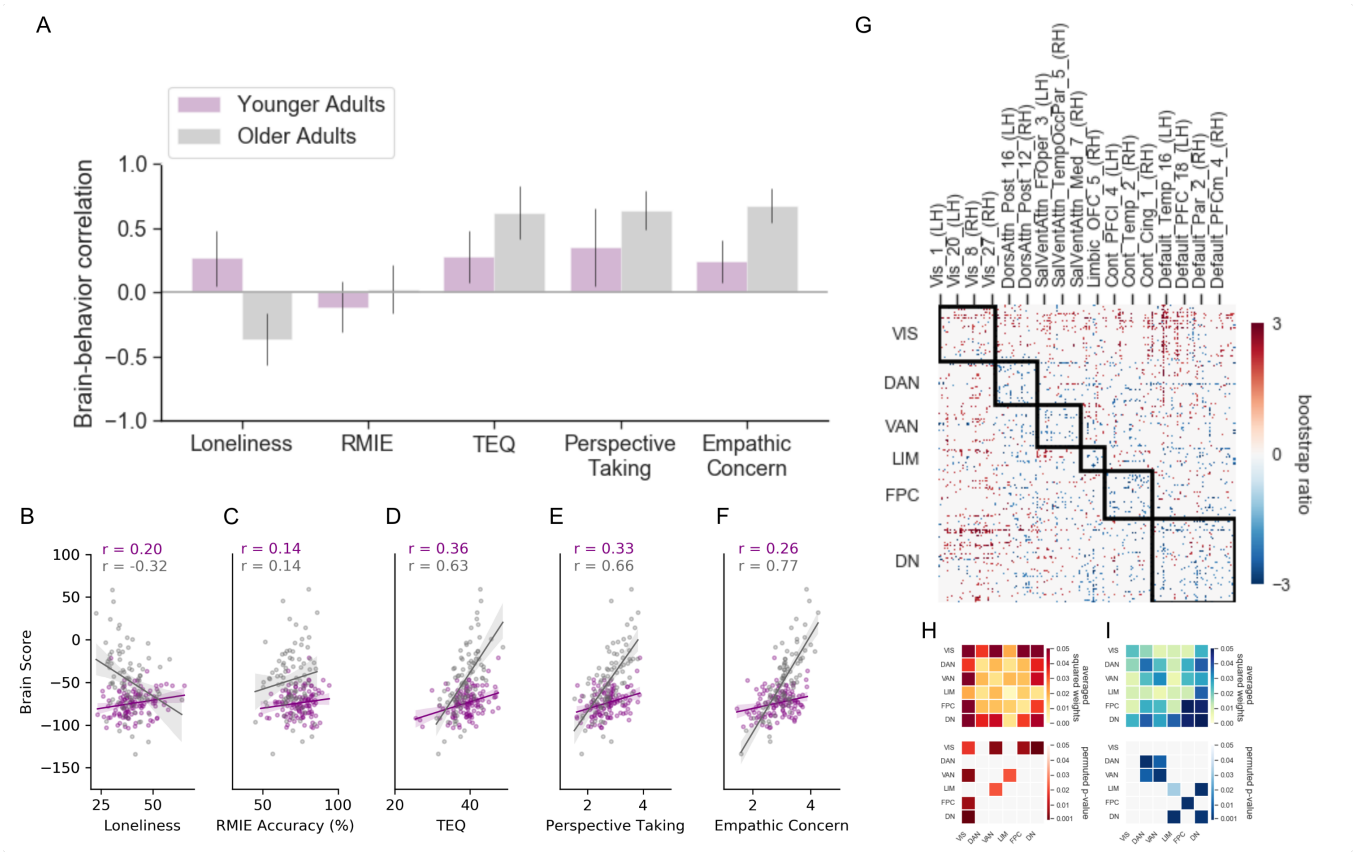


Table 4.3: Caption. PLS analysis of brain-behavior covariance for LV1. (A) Displays the correlation between behavioral loneliness, empathic responding measures, and RSFC in younger and older adults. Error bars show 95% confidence intervals determined by bootstrap resampling. Scatterplots in panels B-F show correlations between participant brain scores and behavioral measures controlling for age, site, gender, education, neuroticism, and cognition as a function of each behavioral measure. (G) Correlation matrix of the reliable pairwise functional connections associated with behavior. The matrix bootstrap ratios are thresholded at $\pm$ 2 to 3. Network-level contributions to the positive (H) and negative (I) connectivity pattern for LV1: Top matrices show the averaged squared salience weights, which reflects a summary of the connectivity pattern; bottom matrices show significant network contribution estimated using permutation testing on the correlation matrix in (G). Behaviors that correlate positively with the pattern are represented in warm colors, and negative brain-behavior correlations in cool colors.

RSFC related to subdomains of empathic responding

A second significant pattern revealed shared and diverging associations related to various facets of empathic responding ($p < 0.01$; 16.53% covariance explained; Figure 4.4). The brain-behavioral correlations for both groups are shown in Figure 4.4A. Across both age groups, better performance on emotional recognition on the RMIE task correlated positively with greater intra- and inter-network RSFC among regions in heteromodal association cortex (Figure 4.4G). This pattern was particularly prominent within the dorsal attention, limbic, frontoparietal, and default networks, and between default to limbic networks and frontoparietal to dorsal attention networks (Figure 4.4H). LV2 also captured a RSFC pattern of age group differences in the relationship between RSFC and all empathic responding measures. Younger adults with higher scores on the TEQ, perspective taking, and empathic concern showed strong intra-network connectivity of the visual network and connectivity between the visual network with the other five networks (Figure 4.4I). No significant associations between RSFC and these three self-reports measures of empathic responding were found in older adults. When controlling for covariates of no interest, significant brain-behavior correlations remained in younger adults for TEQ, perspective taking, and empathic concern (Supplementary Table 4.S2). For older adults, a significant positive brain-behavior correlation emerged for perspective taking. Furthermore, when controlling for social network size an additional positive brain-behavior correlation emerged for empathic concern in older adults. Scatterplots of the relationship between the corrected brain scores and each behavioral measure can be found in (Figure 4.4B-F).

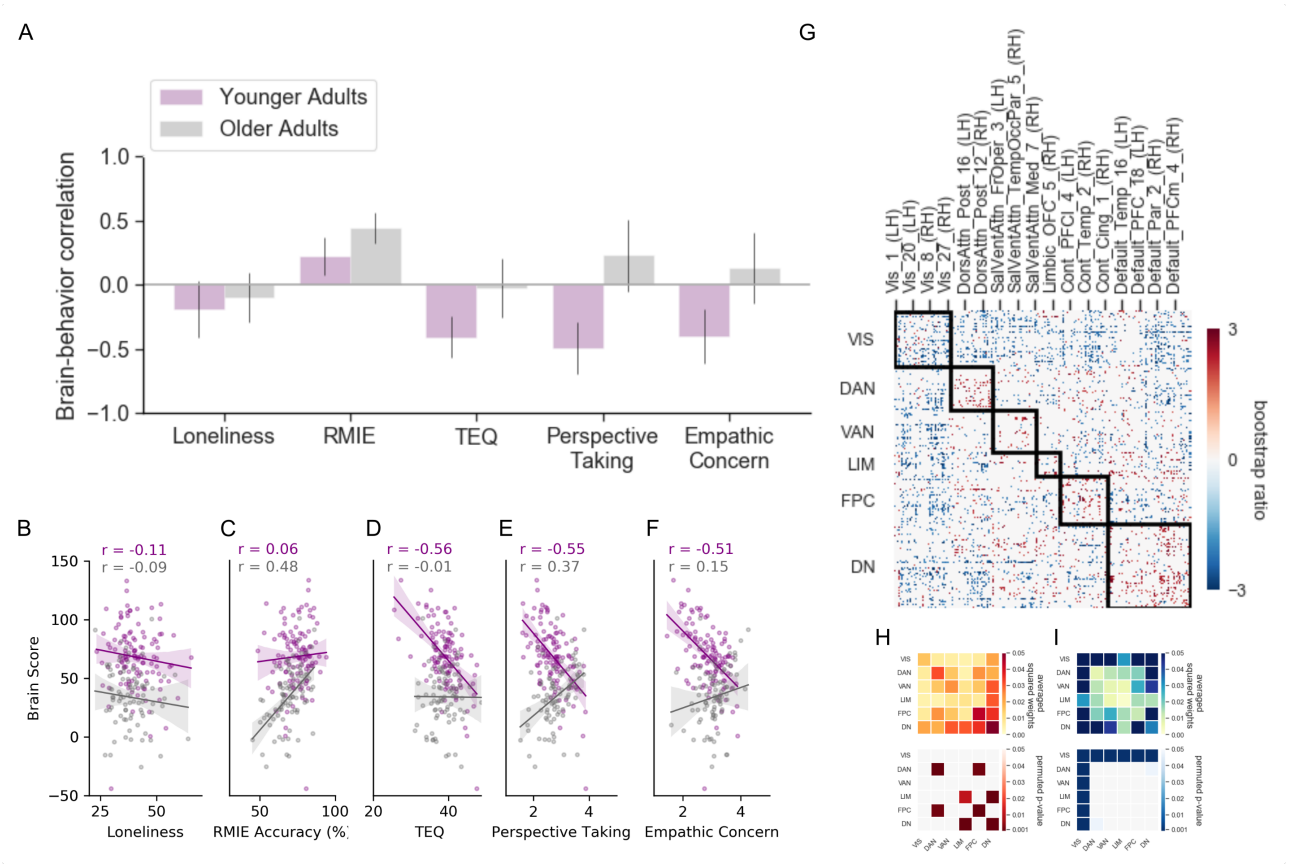


Table 4.4: Caption. PLS analysis of brain-behavior covariance for LV2. (A) Displays the correlation between behavioral loneliness, empathic responding, and RSFC in younger and older adults. Error bars show 95% confidence intervals determined by bootstrap through bootstrap resampling. (B-F) Scatterplots in panels B-F show correlations between participant brain scores and behavioral measures controlling for age, site, gender, education, neuroticism, and cognition as a function of each behavioral measure. (G) Correlation matrix of the reliable pairwise functional connections associated with behavior. The matrix bootstrap ratios are thresholded at $\pm$ 2 to 3. Network-level contributions to the positive (H) and negative (I) connectivity pattern for LV1: Top matrices show the averaged squared salience weights, which reflects a summary of the connectivity pattern; bottom matrices show significant network contribution estimated using permutation testing on the correlation matrix in (G). Behaviors that correlate positively with the pattern are represented in warm colors and negative brain-behavior correlations in cool colors.

4.6.4 Cross-validation of PLS results

To account for overfitting from our PLS analysis, we conducted a second analysis to assess the stability of the identified patterns (see Methods for more details). A 5-fold cross-validation was performed on the two LVs by correlating the RSFC-behavior associations of each LV in the training set and calculating the mean correlation across folds. The mean correlation was strongly correlated (r = 0.54). RSFC-behavior correlations in the test set representing 20% of the sample for the test set were lower but remained significantly correlated across fold (r = 0.19; p = 0.003), suggesting that PLS LVs estimated from train data were stable in the testing set.

4.7 Discussion

We explored the relationship between loneliness, empathic responding, and RSFC in younger and older adults to delineate differences in sociality and brain function associations. Older adults reported feeling less lonely and expressed greater self-reported empathy. However, they scored lower on a performance-based measure of emotional recognition (RMIE). Negative associations between loneliness and empathy were observed across the lifespan, with more robust associations detected for older versus younger adults. Predicted age differences were observed in the association between loneliness, empathic responding, and RSFC. Brain and behavioral associations did not differ for loneliness and empathy in younger adults. Positive associations were observed for both aspects of sociality and RSFC between visual regions and spatially distributed brain systems. Older adults showed a divergence in RSFC associations between loneliness and empathy. Higher self-reported loneliness in older adults was associated with greater RSFC within heteromodal association networks and between attention (dorsal and ventral) and the default and frontoparietal and limbic networks. In contrast, and consistent with younger adults, higher self-reported empathy for older adults was associated with greater visual network connectivity to the cortex. These findings adjudicate previous reports (Mwilambwe-

Tshilobo et al., 2019; Spreng et al., 2020) and reveal that sociality and RSFC associations differ for young and older adults. Our findings also show that age differences are specific to loneliness and involve cortical association networks related to internally-directed cognition and socioemotional processing.

4.7.1 Age differences in RSFC related to loneliness

We observed a difference in the relationship between RSFC and loneliness between younger and older adults. Integration of visual and association networks was related to higher loneliness in young. In contrast, higher loneliness in older adults was marked by lower RSFC of visual regions and greater intra- and inter-network RSFC among higher-order association networks. These findings support our hypothesis that age-related differences in the association between loneliness and brain function reflect a shift from externally- to internally-oriented processing regions, reconciling previous reports (Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020). Importantly, no shared pattern relating loneliness to RSFC was observed between age groups, suggesting a qualitatively different pattern in the neural basis of loneliness across the lifespan. Although we could not test this directly, we suggest that these differences reflect a shift in the perception and experience of loneliness into older age.

In younger adults, integration of visual and association networks may reflect increased social perception demands to monitor for threatening social cues or seek new opportunities for social connection (J. Cacioppo & Hawkley, 2009). In contrast, for older adults, functional segregation of the visual network and increased integration within and between higher-order association networks related to loneliness may reflect a shift towards more internally-directed processing (cf. Spreng et al., 2020), consistent with an age-related shift towards prioritizing socioemotional goals. Instead of searching for new social contacts, older adults have smaller social networks that prioritize close social connections (L. Carstensen, 1992). As the pursuit of new social experiences declines with age, lonely older adults may rely more on reminiscing about past experiences (Ross & Inagaki, 2022)

or mentalizing about future social engagements (Spreng et al., 2020). Autobiographical recollection and future thinking are robustly related to the default network and its interactions with other association networks (Andrews-Hanna et al., 2014; Schacter et al., 2012) which closely converges with the connectivity pattern associated with loneliness in older but not younger adults in our study. In the context of previous reports (Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020), our findings suggest that the experience of loneliness shifts over the adult lifespan. However, given that our study was cross-sectional, an alternative explanation for the age differences found could be due to older adults experiencing loneliness more chronically than younger adults. Future studies are necessary to examine the experience of loneliness and associated cognitive, social, and neural antecedents and sequelae into older age (Bzdok & Dunbar, 2020; Spreng & Bzdok, 2021).

Another possible explanation for the age differences in RSFC associated with loneliness is that the healthy aging is characterized by brain network dedifferentiation (Chen et al., 2014; Malagurski et al., 2020; Setton et al., 2022). Dedifferentiation in older adulthood may, in part, compensate for functional reorganization of the aging brain (Reuter-Lorenz Cappell, 2008), although some aspects of dedifferentiation are also associated with declining brain health, such as the accumulation of white matter hyperintensities (Kantarovich et al., 2022). However, unlike healthy aging our findings in lonely older adults indicate greater within-network connectivity in higher-association networks. We previously reported reduced network modularity associated with loneliness in younger adults (Mwilambwe-Tshilobo et al., 2019). While speculative, lonely older adults may compensate for these age and loneliness related functional changes by increasing within-network connectivity of higher-association networks. Future research would benefit from disentangling the combined effects of aging and loneliness on brain functional reorganization.

Finally, our two earlier studies reported divergent associations between loneliness and brain function for younger and middle-aged adults. Interestingly, we only partially replicated the findings from our study involving a sizeable middle-aged cohort drawn from

the UK Biobank ((Spreng et al., 2020). Consistent with Spreng et al. (2020), loneliness in older adults was related to greater RSFC within the default, frontoparietal control and limbic networks, and associations between the default and limbic network. Unlike Spreng et al. (2020), this pattern extended to greater connectivity within and among ventral and dorsal attention networks in our older adult cohort, indicating that the impact of loneliness on brain function may continue to shift beyond midlife into older age. Our findings provide further evidence that the UK Biobank, representing a large population-based cohort, is a developmentally unique sample (Kiesow et al., 2021) that may not capture brain and behavioral associations observed in early or late adulthood. Future research including an adult lifespan sample is needed to fully characterize differences in loneliness and brain associations across the broad continuum of adult human development.

4.7.2 Shared RSFC pattern related to empathy across age groups

We observed age-invariant associations between subdomains of empathy and RSFC, characterized by greater interactions within the visual network and connections with ventral attention, frontoparietal control, and default networks. We did not predict this robust age-invariant association given limited evidence relating visual network functioning to empathy (Katsumi et al., 2021; Schurz et al., 2021). We speculate that the dependence of empathic ability on the perception of social cues (J. Cacioppo & Hawkley, 2009) may underlie the neural patterns observed here.

Few studies have examined the neural correlates of empathy in aging. Decreased activation in the insula and anterior/mid-cingulate (core nodes of the ventral attention network; (Chen et al., 2014; Riva et al., 2018) have been associated with affective empathy in older adults. However, recent work failed to find similar age differences (Ziaei et al., 2021). More robust age-related brain differences have been observed for cognitive empathy, specifically implicating the dorsal medial prefrontal cortex, a key node of the default network related to social cognition ((Beadle & de la Vega, 2019; Moran et al., 2012). Our observations suggest differing age-related trajectories in empathy and loneli-

ness. The association between empathic functioning and RSFC was age-invariant. In contrast, the association between loneliness and RSFC differed with age, as we report here (Figure 3) and in other age cohorts (e.g., Mwilambwe-Tshilobo et al., 2019; Spreng et al., 2020).

4.7.3 Differences in RSFC across dimensions of empathic responding

We included performance-based emotional recognition (RMIE) and self-reported trait empathy measures, allowing us to examine how these different expressions of empathic responding relate to RSFC (Ziaei et al., 2021). As revealed in the second LV (Figure 4), these two aspects of empathic responding are associated with divergent neurocognitive systems, consistent with previous reports that empathic responding encompasses affective and cognitive processes (Christov-Moore et al., 2020; Schurz et al., 2021). Specifically, we identified an age-invariant difference in the relationship between emotion recognition ability using the RMIE task and self-reported trait empathy measures. Younger and older adults shared a common RSFC pattern associated with better performance on the RMIE characterized by greater intra- and inter-network connectivity of association networks. In contrast, self-reported trait empathy measures in younger adults were associated with greater visual network connectivity with the rest of the brain. This divergence in intrinsic network connectivity patterns may reflect functional organizational features of brain networks that enable specialized and flexible social cognitive functioning. Recent work on brain network interactions related to social cognitive functioning by Schurz et al. (2020) proposes that differences in network segregation and integration may account for differences in connectivity patterns across theory of mind and empathy tasks. Specifically, interactions between the default network and attention and frontoparietal networks. However, further evidence is needed to disambiguate differences in network interactions underlying task versus trait-based measures of empathic responding.

4.7.4 Behavioral associations between loneliness and empathy

Finally, while not a central aim of the current study, our behavioral findings confirmed previous reports of an inverse association between loneliness and empathy (Nakagawa et al., 2015) observed in younger and older adults (Beadle et al., 2012). While we used a different self-report measure to assess affective and cognitive aspects of empathy (TEQ versus Empathy Quotient), we also observed a negative association across the entire sample. However, when we examined the relationship between loneliness and subdomains of empathic responding separately in each age group, reliable associations were only observed for older adults on self-report measures assessing the affective features of empathy. This finding may reflect shifts in motivational goals that occur as people age. Socioemotional selectivity theory states that socioemotional goals become salient for older adults (L. L. Carstensen, 2006). This change in goal hierarchies in later life shifts cognitive resources towards emotional regulation to meet heightened socioemotional needs (Mather, 2016; Mather & Carstensen, 2005). Thus, the relationship between loneliness and empathy may be heightened in older adulthood, reflecting the importance of maintaining adaptive socioemotional functioning in late-life development. Our results further underscore this point by demonstrating that aspects of relationship quality are important factors to consider when investigating age differences related to loneliness. We show that loneliness was inversely related to the quality of social relationships, and that controlling for social support and friendship attenuated the association between loneliness and RSFC in younger adults, but not older adults.

4.7.5 Conclusion

Loneliness is a modifiable risk factor associated with various health problems in older adulthood (Ong et al., 2016). Further, the experience of loneliness is negatively related to empathic responding, which is necessary for fostering and maintaining close relationships (Morelli et al., 2017), essential for well-being (Beadle & de la Vega, 2019), and critical

for increasing personal importance in later life (L. Carstensen, 1992). Here we examined associations between these essential dimensions of sociality, brain function, and differences with age. Our findings revealed that the negative association between loneliness and empathy, observable across the lifespan, was greater in older adults. While longitudinal studies are needed to determine causal associations, it is possible that experiencing loneliness in later life may precipitate a cascade of adverse changes in social functioning that could exacerbate feelings of social isolation. We also identified a pattern of age differences in brain function that is differentially related to loneliness and empathy in older, but not younger, adults. Extending our previous work in young (Mwilambwe-Tshilobo et al., 2019) and middle-aged (Spreng et al., 2020) cohorts, the current results demonstrate that loneliness impacts different neurocognitive systems across the adult lifespan. Early theoretical accounts implicating loneliness in disordered social perception (J. Cacioppo & Hawkley, 2009) may not fully capture the experience of loneliness in later life. Lower motivation to form new social bonds and access to a larger store of lived social experiences may shift the impact of loneliness towards more internally-directed cognitive processes and associated neural networks, as older adults mentalize and reminiscence to fulfill unmet social desires. Whether and how such a shift may precipitate the adverse cognitive sequelae associated with loneliness in later life is an important direction for future research (Bzdok & Dunbar, 2020; Spreng & Bzdok, 2021).

4.8 Data availability statement

All data from the current report are open access and publicly available (see (Spreng et al., 2022), for data descriptor). Demographic and behavioral data are available within the Open Science Framework project "Goal-Directed Cognition in Older and Younger Adults" (http://osf.io/yhzxe/); neuroimaging data are available on OpenNeuro (https://openneuro.org/datasets/ds003592).

4.9 Author contributions

Laetitia Mwilambwe-Tshilobo: Conceptualization; Data curation; Formal analysis; Investigation Methodology; Visualization; Writing-original draft. Roni Setton: Data curation; Writing-Review & editing. Danilo Bzdok: Funding acquisition; Writing-Reviewing & Editing. Gary Turner: Funding acquisition; supervision of data collection; Writing-Reviewing & Editing. R. Nathan Spreng: Conceptualization; Funding acquisition; Supervision; Writing-Review & editing.

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4.12 Supplemental Results

Behavioral and RSFC associations controlling for quantity and quality of social relationships

There is a possibility that aspects of social relationship quantity and quality may differentially impact the effects of loneliness across age groups. Therefore, we wanted to examine the impact of objective and subjective characteristics of social networks in younger and older adults. Two separate partial correlation analyses were performed using social network size (quantity) and subjective perception of social support and friendship were included as additional covariates (quality) as additional covariates for each LV. For LV1, partial correlations between the PLS brain scores and behavioral measures remain significant when social network size was included as a covariate (top section of Supplementary Table 4.S3). When measures of relationship quality were included as covariates, all brain-behavior associations remained significant except for the correlation between RSFC and loneliness in younger adults which was trending (pr = 0.07; top section of Supplementary Table 4.S4). For LV2, when controlling for social network size an additional positive brain-behavior correlation emerged for empathic concern in older adults (bottom section of Supplementary Table 4.S3). When social support and friendship measures were included as covariates only perspective taking remained significant from the primary PLS results. However, the brain score- RMIE association became significant (bottom section of Supplementary Table 4.S3).

Characterizing associations between RSFC, loneliness, and empathic functioning

The RSFC data used in the primary PLS results focused on six networks: visual, dorsal attention, ventral attention, limbic, frontoparietal control, and default. To prove a comprehensive assessment of the PLS results using whole-brain RSFC, we included the so-

[1]To account for the possibility that neuroticism may confound the age effects observed in our PLS analysis we ran the correlations excluding neuroticism. The results shown here remained consistent.

[2]Results remained significant when excluding neuroticism demonstrating that the age effects are not confounded by neuroticism.

Table 4.S1: Correlation of UCLA loneliness with empathic responding measures in younger and older adults

Table 1A

Empathy Measure	Younger Adults (n=128)	Older Adults (n=92)	Full Sample (n=220)
RMIE	0.05 (0.60) [-0.12, 0.22]	0.07 (0.5) [-0.13, 0.27]	0.07 (0.41) [-0.09, 0.22]
TEQ	-0.03 (0.67) [-0.12, 0.14]	-0.27 (0.009)* [-0.45, -0.07]	-0.12 (0.14) [-0.27, 0.04]
Perspective Taking	-0.07 (0.44) [-0.24, 0.11]	-0.31 (0.003)* [-0.48, -0.11]	-0.16 (0.04)* [-0.31, -0.01]
Empathic Concern	-0.03 (0.73) [-0.2, 0.14]	-0.33 (0.001)* [-0.5, -0.14]	-0.20 (0.01)* [-0.34, -0.05]

Table 1B

Empathy Measure	Younger Adults (n=118)	Older Adults (n=87)	Full Sample (n=205)
RMIE	0.10 (0.30) [-0.09, 0.28]	0.10 (0.39) [-0.12, 0.31]	0.08 (0.25) [-0.6, 0.22]
TEQ	-0.08 (0.40) [-0.26, 0.11]	-0.22 (0.05)* [-0.42, -0.0]	-0.15 (0.04)* [-0.28, -0.01]
Perspective Taking	0.04 (0.65) [-0.14, 0.23]	-0.22 (0.05) [-0.48, 0.0]	-0.05 (0.46) [-0.19, 0.09]
Empathic Concern	-0.03 (0.73) [-0.22, 0.15]	-0.22 (0.05)* [-0.42, -0.0]	-0.09 (0.19) [-0.23, 0.05]

Table 1C

Empathy Measure	Younger Adults (n=89)	Older Adults (n=70)	Full Sample (n=159)
RMIE	0.05 (0.65) [-0.17, 0.26]	0.08 (0.55) [-0.17, 0.32]	0.06 (0.46) [-0.1, 0.22]
TEQ	-0.03 (0.82) [-0.24, 0.19]	-0.17 (0.19) [-0.39, 0.08]	-0.09 (0.25) [-0.25, 0.07]
Perspective Taking	0.16 (0.14) [-0.05, 0.37]	-0.18 (0.17) [-0.4, 0.07]	0.03 (0.72) [-0.13, 0.19]
Empathic Concern	0.04 (0.74) [-0.18, 0.25]	-0.19 (0.13) [-0.42, -0.06]	-0.05 (0.58) [-0.2, 0.11]

Table 1D

Empathy Measure	Younger Adults (n=117)	Older Adults (n=86)	Full Sample (n=203)
RMIE	0.15 (0.13) [-0.04, 0.33]	0.22 (0.05) [-0.0, 0.42]	0.15 (0.04) [0.1, 0.28]
TEQ	-0.01 (0.94) [-0.2, 0.18]	-0.17 (0.13) [-0.38, 0.05]	-0.10 (0.17) [-0.24, 0.04]
Perspective Taking	0.11 (0.27) [-0.08, 0.29]	-0.08 (0.50) [-0.29, 0.15]	0.05 (0.49) [-0.09, 0.19]
Empathic Concern	-0.12 (0.20) [-0.3, 0.07]	-0.07 (0.55) [-0.29, 0.16]	-0.07 (0.31) [-0.21, 0.07]

Note: Correlation and partial correlation values between UCLA loneliness and empathic functioning in younger and older adults. p-values in parentheses and 95% confidence intervals in square brackets. Table 1A: Full product-moment correlations within the young and older adult cohorts. Table 1B: Relationship between UCLA loneliness and empathic responding, controlling for gender, site, education, neuroticism, and cognitive composite score. Table 1C: Relationship between UCLA loneliness and empathic responding, controlling for gender, site, education, neuroticism, cognitive composite score and social network size. Table 1D: Relationships between UCLA loneliness and empathic responding controlling for gender, site, education, neuroticism, cognitive composite score, instrumental support, emotional support, and friendship. For the full sample in all Tables 1B-D, age is controlled for. Note the lower sample size with increasing number of covariates due to missing data. TEQ= Toronto Empathy Questionnaire; RMIE= Reading the Mind in the Eyes Task. * $p< 0.05$; ** $p < 0.01$. *** $p < 0.001$

matosensory network. Although the patterns identified were similar to the primary PLS results, only LV2 remained significant (LV1: p = 0.12; 25.06% covariance explained ; LV2: p = 0.01; 16.22% covariance explained).

Table 4.S2: Correlation between PLS brain scores and behavioral measures with covariates

Group	Behavior	n	r	CI95%	p-value[1]
			LV 1		
Younger Adults	Loneliness	118	0.2	[0.01, 0.37]	0.04
Younger Adults	RMIE	118	0.14	[-0.04, 0.32]	0.13
Younger Adults	TEQ	118	0.36	[0.19, 0.51]	<0.001
Younger Adults	PT	118	0.33	[0.15, 0.49]	<0.001
Younger Adults	EC	118	0.26	[0.08, 0.43]	0.01
Older Adults	Loneliness	87	-0.32	[-0.51, -0.11]	<0.001
Older Adults	RMIE	87	0.14	[-0.08, 0.35]	0.21
Older Adults	TEQ	87	0.63	[0.47, 0.74]	<0.001
Older Adults	PT	87	0.66	[0.52, 0.77]	<0.001
Older Adults	EC	87	0.77	[0.67, 0.85]	<0.001
			LV2		
Younger Adults	Loneliness	118	-0.11	[-0.29, 0.07]	0.23
Younger Adults	RMIE	118	0.06	[-0.13, 0.24]	0.55
Younger Adults	TEQ	118	-0.56	[-0.68, -0.42]	<0.001
Younger Adults	PT	118	-0.55	[-0.67, -0.41]	<0.001
Younger Adults	EC	118	-0.51	[-0.63, -0.36]	<0.001
Older Adults	Loneliness	87	-0.09	[-0.3, 0.13]	0.42
Older Adults	RMIE	87	0.48	[0.29, 0.63]	<0.001
Older Adults	TEQ	87	-0.01	[-0.22, 0.21]	0.96
Older Adults	PT	87	0.37	[0.17, 0.55]	<0.001
Older Adults	EC	87	0.15	[-0.07, 0.36]	0.17

Note: Covariates include age, gender, site, education, neuroticism, and cognition composite score. Participants with missing data on one more of the covariates were omitted from the brain-behavior correlation analysis. TEQ= Toronto Empathy Questionnaire; RMIE= Reading the Mind in the Eyes Task; PT = Perspective Taking; EC= Empathic Concern.

Impact of Age on RSFC

The PLS results captured an age-dependent differences in association between loneliness and RSFC for LV1, and an age-independent association between subdomains of empathy and RSFC. Although we control for age in the partial correlations between RSFC and behaviors, we wanted to rule out the possibility that this result was not due to age loading strongly onto LV1. To assess this, age was partialled out from the original **X** (RSFC) and **Y** (behavior) matrices and repeated the PLS analysis. Similar to the initial analysis, LV1 was significant and captured an age difference in RSFC related to loneliness between younger and older adults (LV1: p = 0.03; explained 25.5% of the covariance; Supplementary Figure 4.S5). LV2 was also significant and captured an age-independent association between

Table 4.S3: Correlation between PLS brain scores and behavioral measures with covariates (including social network size)

Group	Behavior	n	r	CI95%	p-value[2]
			LV 1		
Younger Adults	Loneliness	89	0.28	[0.07, 0.47]	0.01
Younger Adults	RMIE	89	0.19	[-0.03 , 0.39]	0.09
Younger Adults	TEQ	89	0.25	[0.04, 0.45]	0.02
Younger Adults	PT	89	0.34	[0.13, 0.52]	<0.001
Younger Adults	EC	89	0.19	[-0.03, 0.39]	0.09
Older Adults	Loneliness	70	-0.30	[-0.51, -0.06]	0.02
Older Adults	RMIE	70	0.19	[-0.06, 0.42]	0.14
Older Adults	TEQ	70	0.59	[0.40, 0.73]	<0.001
Older Adults	PT	70	0.68	[0.52, 0.79]	<0.001
Older Adults	EC	70	0.77	[0.64, 0.85]	<0.001
			LV2		
Younger Adults	Loneliness	89	-0.19	[-0.39, 0.03]	0.08
Younger Adults	RMIE	89	0.04	[-0.18, 0.26]	0.72
Younger Adults	TEQ	89	-0.58	[-0.71, -0.41]	<0.001
Younger Adults	PT	89	-0.54	[-0.68, -0.37]	<0.001
Younger Adults	EC	89	-0.47	[-0.63, -0.29]	<0.001
Older Adults	Loneliness	70	-0.13	[-0.37, 0.12]	0.3
Older Adults	RMIE	70	0.51	[0.30, 0.67]	<0.001
Older Adults	TEQ	70	0.11	[-0.14, 0.35]	0.39
Older Adults	PT	70	0.44	[0.22, 0.62]	<0.001
Older Adults	EC	70	0.34	[0.10, 0.54]	0.01

Note: Covariates include age, gender, site, education, neuroticism, cognition composite score, and social network size. TEQ= Toronto Empathy Questionnaire; RMIE= Reading the Mind in the Eyes Task; PT = Perspective Taking; EC= Empathic Concern.

RSFC and empathy (LV2: p = 0.006; 17.92% covariance explained; Supplementary Figure 4.S6).

Brain-behavior correlations remained largely unchanged for both LVs. The most notable differences from the original results emerged for LV1 at the network level (Supplementary Figure 4.S5 H-I). The positive expression of LV1 was driven solely by the connectivity of the visual network, and the negative expression of LV included an additional network interaction between the frontoparietal and dorsal attention networks. The relationship between loneliness and RSFC was still age-independent; however, unlike in the primary analysis, the correlation for older adults was reliable. Supplementary Figure 4.S7 shows the covariance of LV1 and LV2 before and after partialling age from the X and Y matrices. If X and Y were dependent on age, we might see a significant decrease in the

Table 4.S4: Correlation between PLS brain scores and behavioral measures with covariates (including social network size)

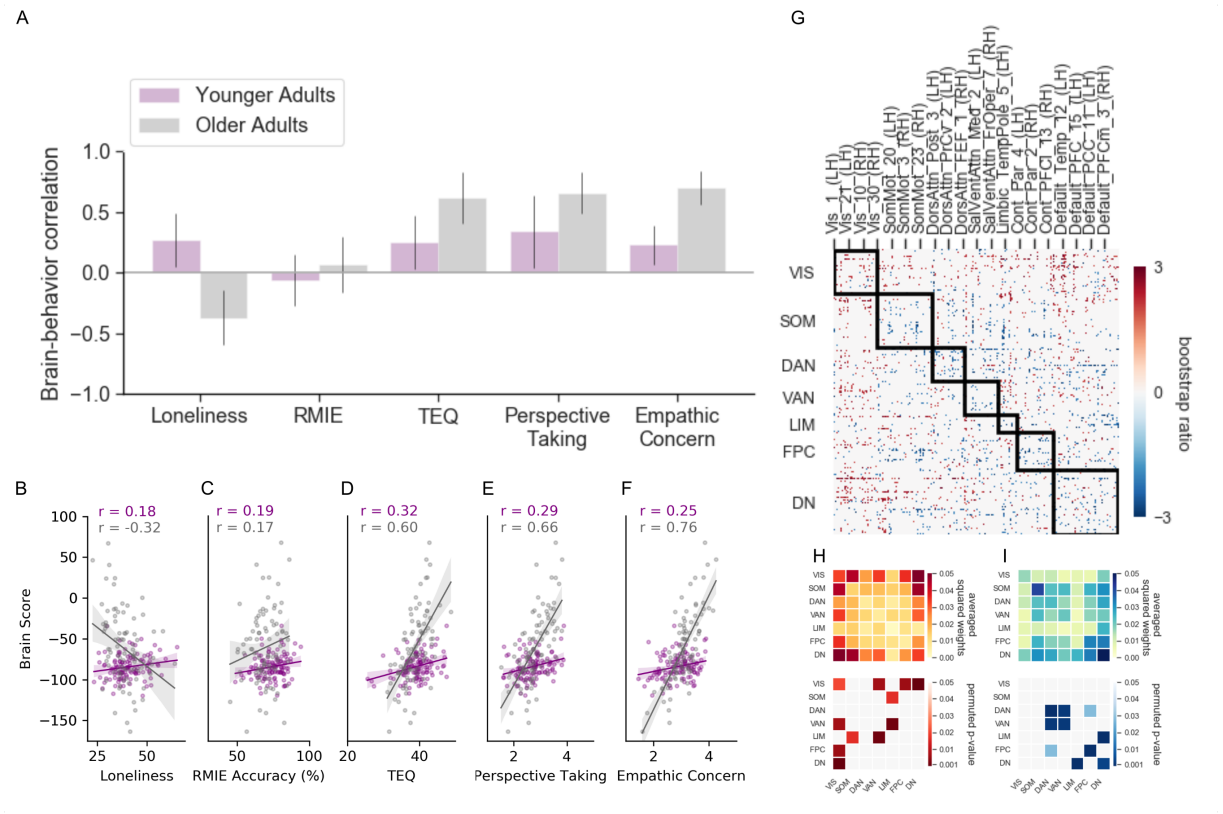
Group	Behavior	n	r	CI95%	p-value
			LV 1		
Younger Adults	Loneliness	117	0.18	[-0.01, 0.35]	0.07
Younger Adults	RMIE	117	0.14	[-0.05, 0.32]	0.16
Younger Adults	TEQ	117	0.38	[0.20, 0.53]	<.001
Younger Adults	PT	117	0.34	[0.16, 0.49]	<.001
Younger Adults	EC	117	0.26	[0.07, 0.43]	0.01
Older Adults	Loneliness	86.0	-0.25	[-0.45, -0.03]	0.03
Older Adults	RMIE	86.0	0.10	[-0.13, 0.32]	0.39
Older Adults	TEQ	86.0	0.62	[0.46, 0.74]	<.001
Older Adults	PT	86.0	0.65	[0.50, 0.76]	<.001
Older Adults	EC	86.0	0.76	[0.65, 0.84]	<.001
			LV2		
Younger Adults	Loneliness	117	-0.17	[-0.35, 0.02]	0.07
Younger Adults	RMIE	117	0.08	[-0.11, 0.26]	0.43
Younger Adults	TEQ	117	-0.56	[-0.68, -0.42]	<.001
Younger Adults	PT	117	-0.55	[-0.67, -0.40]	<.001
Younger Adults	EC	117	-0.52	[-0.64, -0.37]	<.001
Older Adults	Loneliness	86.0	0.02	[-0.21, 0.24]	0.88
Older Adults	RMIE	86.0	0.46	[0.26, 0.62]	<.001
Older Adults	TEQ	86.0	-0.04	[-0.26, 0.19]	0.73
Older Adults	PT	86.0	0.33	[0.11, 0.51]	<.001
Older Adults	EC	86.0	0.11	[-0.11, 0.33]	0.32

Note: Impact of relationship quality on RSFC-behavior associations. Covariates include age, gender, site, education, neuroticism, cognition composite score, emotional support, instrumental support, and friendship. The last three covariates were included as measures of participants subjective perception of the social support and companionship available to them. The PLS results remain significant in both LVs except for the correlation between PLS brain scores and loneliness in younger adults (LV1). TEQ= Toronto Empathy Questionnaire; RMIE= Reading the Mind in the Eyes Task; PT = Perspective Taking; EC= Empathic Concern.

effect size for LV1 after partialling out age. We did not observe this in, suggesting that our primary for LV2 are not due to age strongly loading to LV1.

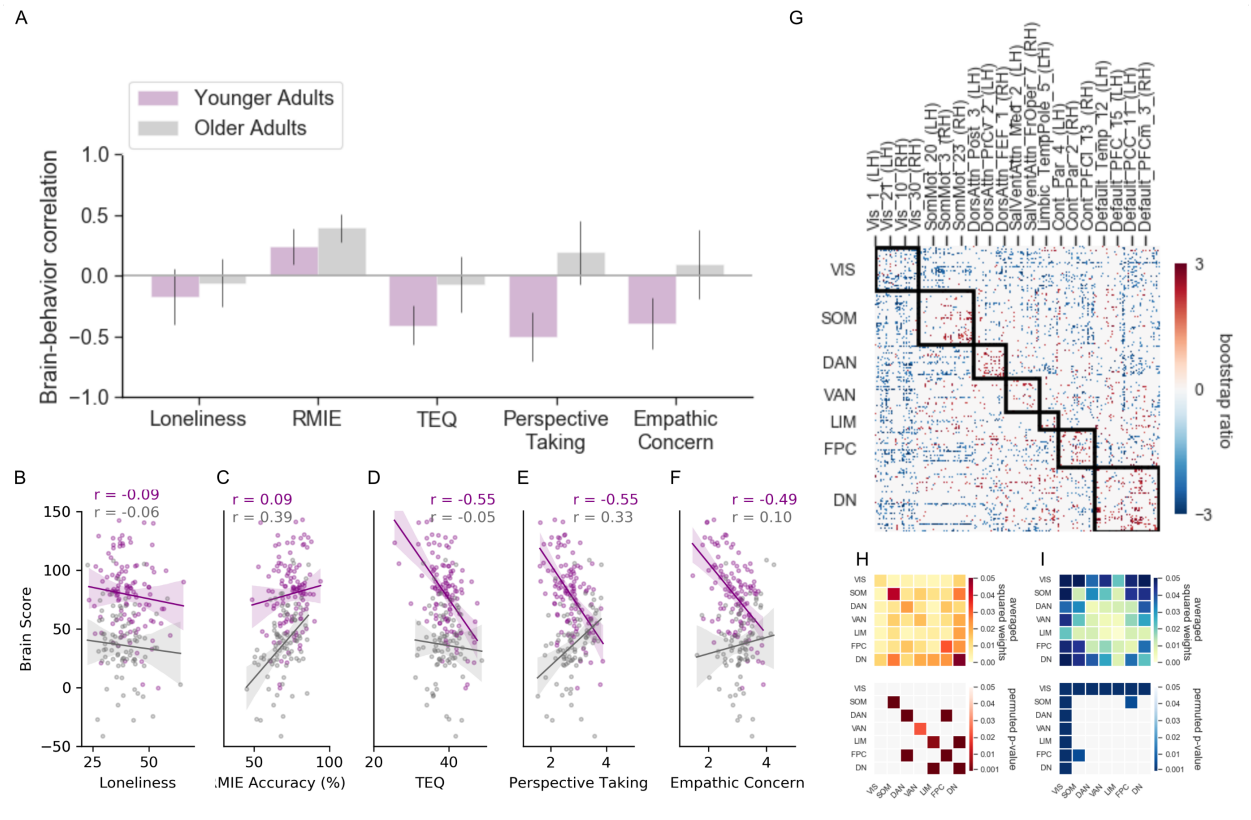
Impact of motion on RSFC

The participants included in the present study were part of a large cohort (n = 301), for which analyses related to the impact of motion on RSFC indicate residual motion effects (Setton Mwilambwe-Tshilobo et al., 2022). Using mean FD calculated from the middle-echo (TE2; before processing), they reported no age differences or interactions with motion when assessing the RSFC data of younger and older adults. To account for the possib-



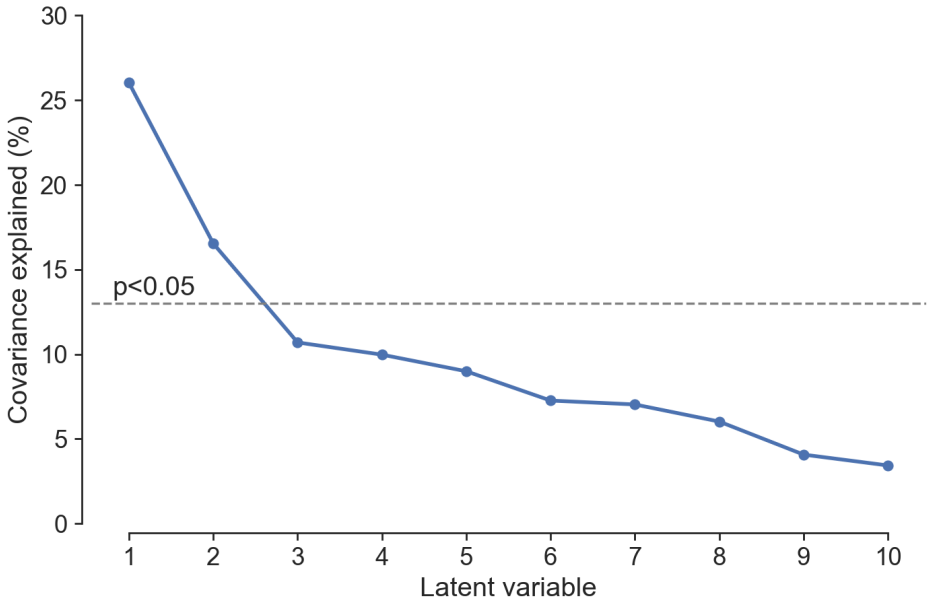
Supplemental Material 4.S1: (A) Displays the correlation between behavioral loneliness, empathic responding measures, and functional connectivity in younger and older adults. Error bars show 95% confidence intervals determined by bootstrap through bootstrap resampling. Scatterplots in panels B-F show participant brain scores corrected for age, site, gender, education, neuroticism, and cognition as a function of each behavioral measures. Whole-brain functional networks are based on Yeo 7-network solution. (G) Correlation matrix of the reliable pairwise functional connections associated with behavior. The matrix bootstrap ratios are thresholded at $\pm$ 2 to 3. Significant contributions of network pairs to the positive (H) and negative (I) connectivity patterns for LV1. Behaviors that correlate positively with the pattern are represented in in red, negative brain-behavior correlations in blue. VIS = visual, SOM = somatomotor, DAN = dorsal attention, VAN = ventral attention, LIM = limbic, FPC = frontoparietal control, DN = default.

ility that residual motion effects did not confound the main findings in the current subset of participants, we first conducted an ANOVA to test for age group and site differences in FD, controlling for site, gender, education, neuroticism, and cognition (Supplementary



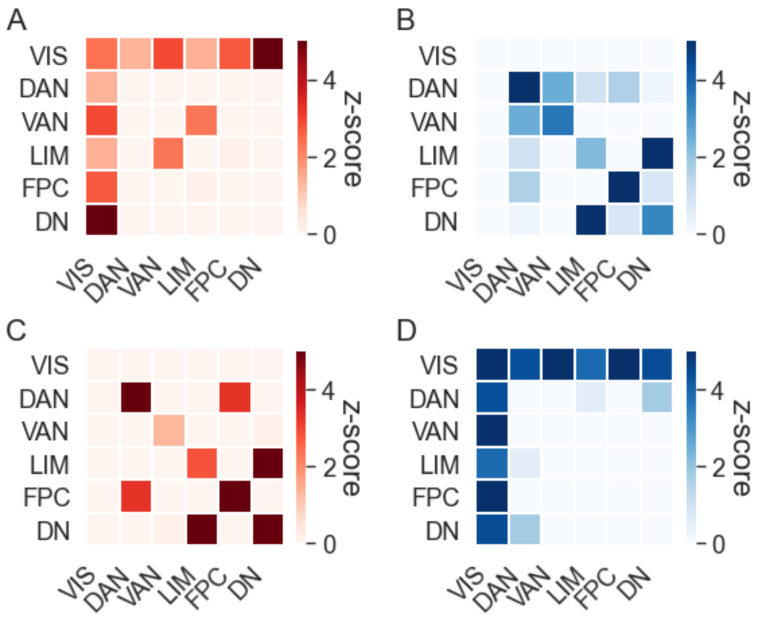
Supplemental Material 4.S2: (A) Displays the correlation between behavioral loneliness, empathy responding, and functional connectivity in younger and older adults. Error bars show 95% confidence intervals determined by bootstrap through bootstrap resampling. Scatterplots in panels B-F show participant brain scores corrected for age, site, gender, education, neuroticism, and cognition as a function of each behavioral measures. Whole-brain functional networks are based on Yeo 7-network solution. (G) Correlation matrix of the reliable pairwise functional connections associated with behavior. The matrix bootstrap ratios are thresholded at $\pm$ 2 to 3. Significant contributions of network pairs to the positive (H) and negative (I) connectivity patterns for LV2. Behaviors that correlate positively with the pattern are represented in in red, negative brain-behavior correlations in blue. VIS = visual, SOM = somatomotor, DAN = dorsal attention, VAN = ventral attention, LIM = limbic, FPC = frontoparietal control, DN = default.

Table 4.S5). Results found a main effects of age group (F(1,216)=13.28, p $<$ .001, p2=.06) and site (F(1,216)=1.39 p = .24, p2= .01), but no interaction (F(1,216)=1.52, p = .22, p2= .01). Follow up t-tests revealed that older adults had higher FD than younger adults (T(219)=



Supplemental Material 4.S3: Scree plot of covariance explained for each latent variable from the partial least-squares (PLS) analysis. Latent variables with covariance values above the dotted line significantly captured the relationship between loneliness and aspects of empathic responding in younger and older adults. The first latent variable explains 26.02% of the variance and the second latent variable explains 16.53%.

-39.89, $p < .001$, [-1.35, -1.24], Cohen's d = 3.7). Next, we conducted a PLS analysis examining the association between RSFC and FD. Similar to the results reported in the larger cohort from which participants were drawn (Setton Mwilambwe-Tshilobo et al., 2022), a significant pattern emerged representing the main effect of motion (LV1: p= 0.02; 57.98% covariance explained; younger adults r=0.84; older adults r =0.82). In addition, we found no significant age group or interactions (LV2: p= 0.47; 42.07% covariance explained), indicating that motion was not a confound in the age group differences. Using the PLS brain scores, we further confirmed that the RSFC pattern that covaried with FD was not associated with age differences in RSFC by performing a partial correlation controlling for gender, education, cognition, and site. Partial correlations between whole-brain RSFC PLS brain scores and FD PLS brain scores were not significant (Supplementary Figure 4.S8).



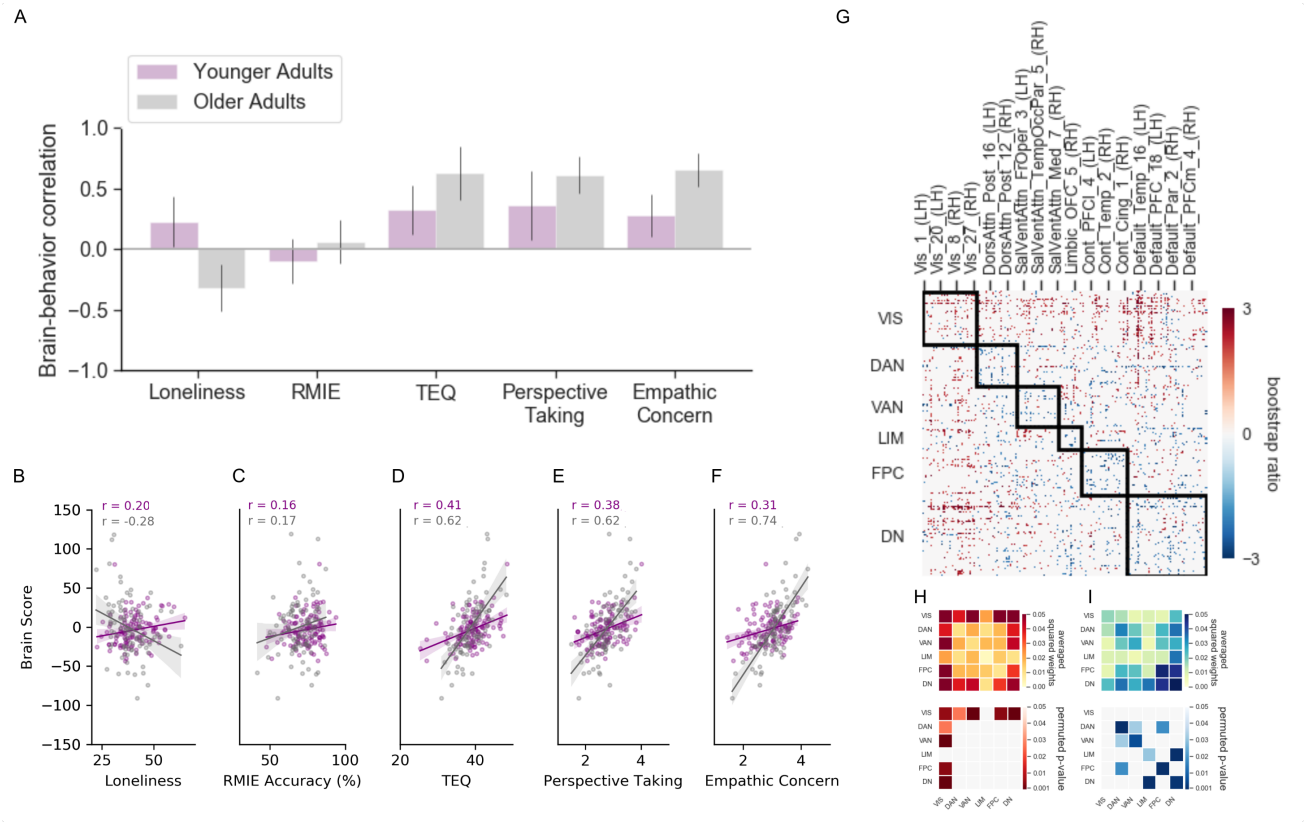
Supplemental Material 4.S4: Network contributions for LV1 and LV2 for the primary PLS analysis expressed as z-scores relative to the permuted null model. The mean contribution is computed for all intra- and inter-network connections. The top row shows the mean (A) positive and (B) negative RSFC pattern for LV1. The matrices in the bottom row are the mean (C) positive and (D) negative RSFC patterns for LV2. Higher values indicate a greater than expected contributions of the network pairs to the respective PLS-derived RSFC pattern for LV1(Figure 3G) and LV2 (Figure 4G).

Finally, to account for the possibility of residual motion impacting the primary PLS results with loneliness and empathic functioning, we included mean FD as a covariate in all analyses, as reported in the main text (Supplementary Table 4.S6). All results held, suggesting they were robust to any residual motion in the data.

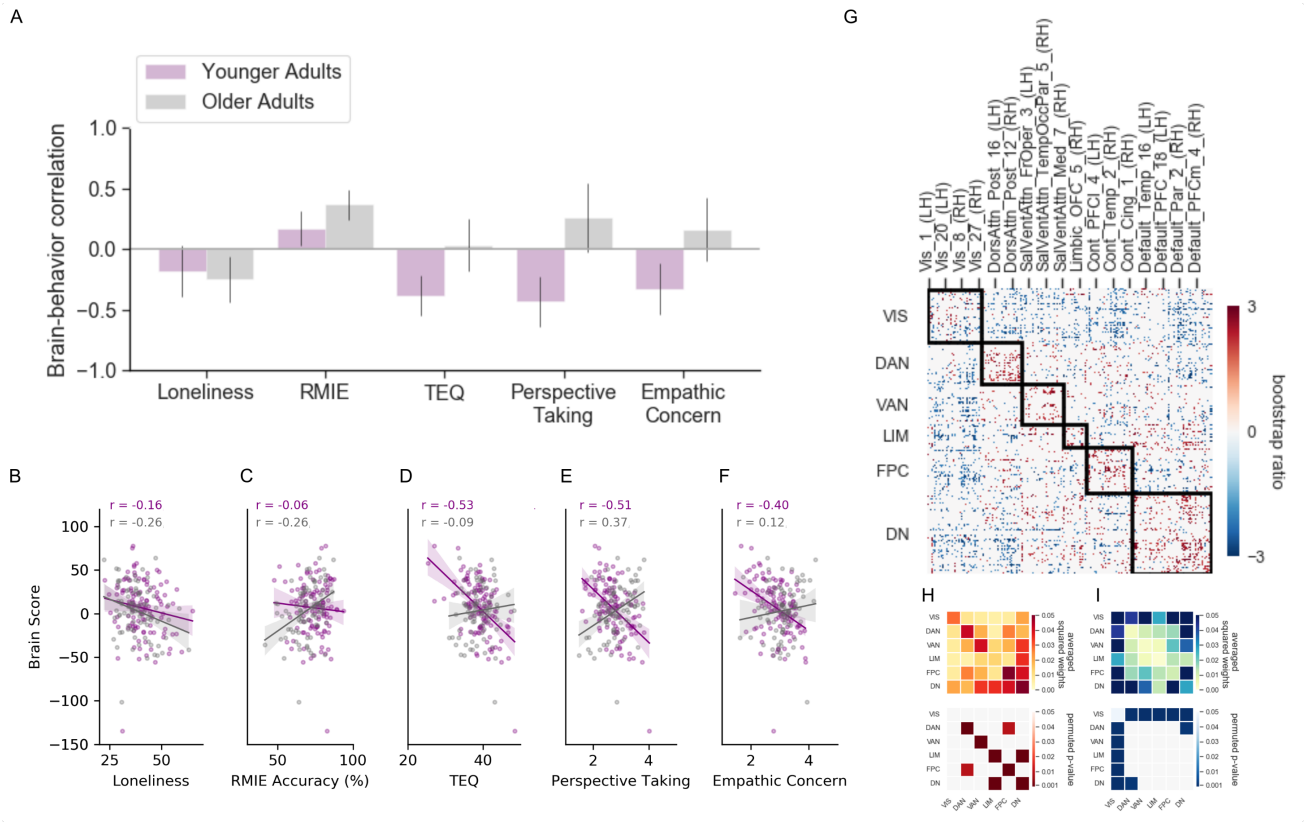
Table 4.S5: ANOVA results for age group and site differences on framewise displacement

Predictor	Sum of Squares	df	Mean Squares	F	p	Partial η^2
			LV 1			
Group	0.04	1	0.040	13.28	<0.001	0.06
Site	0.04	1	0.004	1.39	0.24	0.01
Group x Site	0.04	1	0.004	1.52	0.22	0.01
Error	0.61	216	0.003			

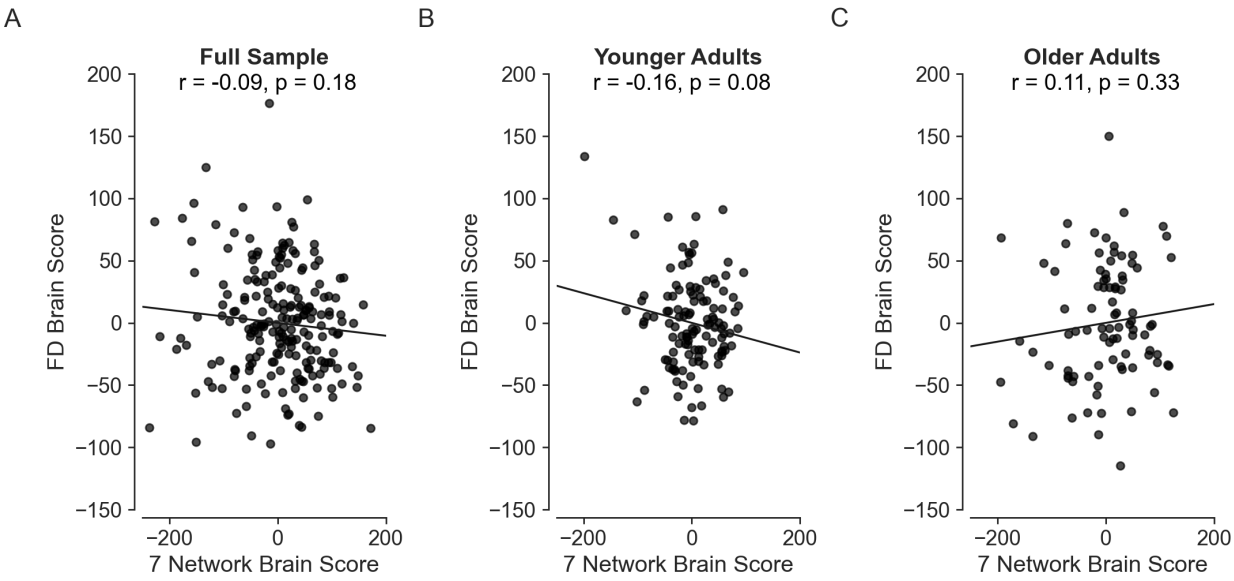
Note: Partial η2 indicates partial eta-squared.



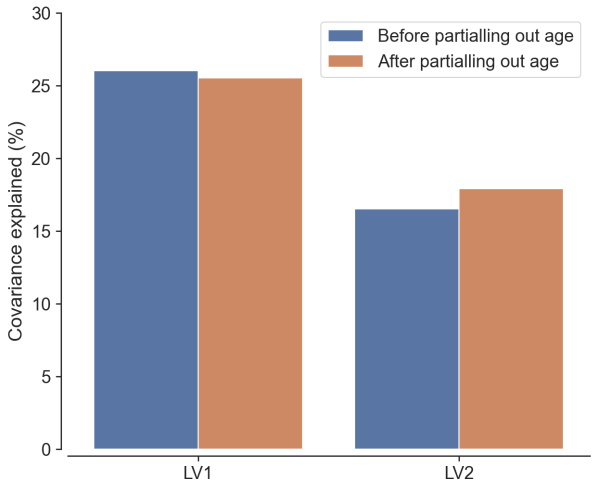
Supplemental Material 4.S5: PLS analysis of covariance between RSFC and behavior controlling for age for LV1. The covariance pattern captured by LV1 were significant (p = 0.03) and accounted for 25.5% of the covariance. (A) Displays the correlation between behavioral measures (loneliness, emotional recognition, and empathic ability) and functional connectivity in younger and older adults. Error bars show 95% confidence intervals determined by bootstrap resampling. Scatterplots in panels B-F show correlation between participant brain scores and behavioral measures controlling for site, gender, education, neuroticism, and cognition. (G) Correlation matrix of the reliable pairwise functional connections associated with behavior. The matrix bootstrap ratios are thresholded at $\pm$ 2 to 3. Significant contributions of network pairs to the positive (H) and negative (I) RSFC patterns for LV1. Behaviors that correlate positively with the pattern are represented in red, negative brain-behavior correlations in blue.



Supplemental Material 4.S6: PLS analysis of covariance between RSFC and behavior controlling for age in LV2. The covariance pattern captured by LV2 was significant (p = 0.006) and accounted for 17.92% of the covariance. (A) Displays the correlation between behavioral measures (loneliness, affective theory of mind, and empathic ability) and functional connectivity in younger and older adults. Error bars show 95% confidence intervals determined by bootstrap resampling. Scatterplots in panels B-F show correlation between participant brain scores and behavioral measures controlling for site, gender, education, neuroticism, and cognition. (G) Correlation matrix of the reliable pairwise functional connections associated with behavior. The matrix bootstrap ratios are thresholded at $\pm$ 2 to 3. Significant contributions of network pairs to the positive (H) and negative (I) RSFC patterns for LV1. Behaviors that correlate positively with the pattern are represented in red, negative brain-behavior correlations in blue.



Supplemental Material 4.S7: Comparison of latent variable covariance before and after partialling out age from the PLS input matrices.



Supplemental Material 4.S8: No relationship between whole-brain PLS brain scores and motion-related PLS brain scores. Framewise displacement (FD) was calculated on the middle echo (TE2) prior to processing. Scatterplots show age differences in whole-brain PLS brain scores on the x-axis and motion-related PLS brain scores on the y-axis (from the six networks included in the primary results) across the (A) full sample, (B) in younger adults, and (C) in older adults

Table 4.S6: Correlation between PLS brain scores and behavioral measures controlling for covariates (including post-processing mean framewise displacement)

Group	Behavior	n	r	CI95%	p-value
			LV 1		
Younger Adults	Loneliness	118	0.2	[0.01, 0.37]	0.03
Younger Adults	RMIE	118	0.14	[-0.05, 0.32]	0.14
Younger Adults	TEQ	118	0.36	[0.19, 0.51]	$<$0.001
Younger Adults	PT	118	0.33	[0.15, 0.49]	$<$0.001
Younger Adults	EC	118	0.26	[0.08, 0.43]	0.005
Older Adults	Loneliness	87	-0.32	[-0.51, -0.11]	0.003
Older Adults	RMIE	87	0.14	[-0.08, 0.35]	0.21
Older Adults	TEQ	87	0.63	[0.47, 0.74]	$<$0.001
Older Adults	PT	87	0.67	[0.57, 0.77]	$<$0.001
Older Adults	EC	87	0.77	[0.67, 0.85]	$<$0.001
			LV2		
Younger Adults	Loneliness	118	-0.11	[-0.29, 0.08]	0.26
Younger Adults	RMIE	118	0.06	[-0.13, 0.24]	0.54
Younger Adults	TEQ	118	-0.56	[-0.68, -0.42]	$<$0.001
Younger Adults	PT	118	-0.55	[-0.67, -0.40]	$<$0.001
Younger Adults	EC	118	-0.51	[-0.63, -0.36]	$<$0.001
Older Adults	Loneliness	87	-0.09	[-0.31, 0.13]	0.42
Older Adults	RMIE	87	0.48	[0.29, 0.63]	$<$0.001
Older Adults	TEQ	87	-0.005	[-0.22, 0.21]	0.96
Older Adults	PT	87	0.37	[0.17, 0.55]	0.001
Older Adults	EC	87	0.15	[-0.07, 0.36]	0.17

Note: Impact of residual motion on RSFC-behavior associations. Covariates include age, gender, site, education, neuroticism, cognition composite score, and post-processing mean FD. TEQ= Toronto Empathy Questionnaire; RMIE= Reading the Mind in the Eyes Task; PT = Perspective Taking; EC= Empathic Concern.

Chapter 5

General Discussion

The current thesis consists of three studies aimed at better understanding the impact of loneliness on the functional network architecture of the brain across the adult lifespan. By employing advanced network neuroscience methods, we characterized the relationship between loneliness and brain network interactions at multiple spatial scales in younger and older adults. The results from these studies provide insight into the neural mechanisms underlying the experience of *state* and *trait* loneliness. In addition, these results emphasize that characterizing the complex interactions among brain regions can provide a more nuanced perspective of how our intrinsic need for social connection influences brain function. Finally, the current findings demonstrate that individual and age-related differences in network connectivity can provide additional insight into the neurocognitive processes that support human social interactions. In this chapter, discusses the main contributions from these studies to the field of social network neuroscience, offer a novel perspective of the neural basis of loneliness, and suggest future directions to address some of the questions raised by the findings presented in this thesis.

The study reported in Chapter 2 characterized distributed brain activity and co-activation patterns during social exclusion using the Cyberball task. Using coordinate-based meta-analytic approaches, we found that social exclusion reliably engages the ventral anterior cingulate and posterior cingulate cortex, inferior and superior frontal gyri, posterior in-

sula, and occipital pole. Contrary to our hypotheses which were grounded in current theories on the neural correlates of social exclusion, we found no reliable activity in the dorsal anterior cingulate a region previously implicated in physical and social pain. Instead, the co-activation patterns of reliable brain regions overlapped with the default network and were associated with social and self-referential cognitive processes. Our findings call into question the theory of a common neural system for physical pain and the affective response to social exclusion. Taken together, the results in Chapter 2 provide new insight emphasizing the role of the default network in the experience of state loneliness.

In Chapter 3, we implemented a novel analytic pipeline to investigate individual differences in the intrinsic functional organization of brain networks related to two psychological constructs related to human sociality–loneliness and meaning in life. In a large cohort of young adults, we found that loneliness and meaning in life were negatively correlated and that the relationship between them was dissociable when examining the functional connectivity of the brain. Individuals who reported less loneliness found life more meaningful and showed greater connectivity between default and limbic networks. In contrast, individuals with higher levels of loneliness showed greater integration of higher-association networks with visual, somatomotor, and ventral attention networks. This connectivity pattern demonstrated novel results linking loneliness with RSFC between neural networks involved in internally and externally oriented processes. These findings suggest that being lonely may drive neurocognitive processes that reorient attentional focus externally to facilitate the detection of negative social cues that characterize trait loneliness.

Finally, Chapter 4 provides preliminary evidence that associations between loneliness and functional network organization change over the adult lifespan. There is accumulating evidence suggesting that loneliness is associated with an increased rate of cognitive decline among older adults (reviewed in Chapter 1), however, very little is known about how loneliness influences brain function in the context of aging. Our results suggest that the association between loneliness and network functional organization shifts

from greater integration of visual regions with higher-order association networks in early adults to greater integration among higher-order association networks in late adulthood. While additional work is needed to expand upon these age-related differences in brain network organization related to loneliness, we hypothesize that these differences may reflect a shift from externally-oriented processing in younger adults to more internally-oriented processing in late adulthood.

The three studies in this thesis use network-based approaches to explore associations between loneliness and functional brain organization across adulthood. In the remaining subsections of this final chapter, I will discuss the common themes and questions that have emerged from this work, and highlight potential avenues for future research.

5.1 Reframing the neural account of loneliness

The neural account of loneliness broadly supports the theoretical view that the perception of social isolation is associated with selective cognitive changes in brain regions implicated in perceptual, affective, attentional, and executive processing of social stimuli. The three studies in the current thesis provide converging evidence that the predominant neural account of loneliness does not fully describe the functional brain alterations associated with loneliness across the adult lifespan. When examining whole-brain or network-level interactions, we get a different understanding than accounts that target individual brain regions in isolation. Individual brain regions are fundamental units of a distributed system of interconnected and interactive parts that collectively give rise to an array of complex human brain functions (Bassett & Sporns, 2017) The utility of leveraging the network neuroscience approach to address social neuroscience questions is rapidly gaining traction (see reviews (Falk & Bassett, 2017; Krendl & Betzel, 2022)). The findings reported in Chapter 2 exemplify the benefits of a network-based approach by demonstrating how adopting an *a priori* focus on specific brain regions may result in an incomplete or possibly

even inaccurate interpretation by overlooking what may be a more reliable coactivation pattern among brain regions.

Interactions among brain regions also vary considerably across individuals (Kong et al., 2019; Wang et al., 2015) and with age (Chen et al., 2014; Geerligs et al., 2015; Setton et al., 2022). The work presented in Chapters 3 and 4 illustrates why considering individual and age differences in brain functional connectivity will be critical as research on the neural correlates of loneliness continues to advance. The study in Chapter 3 adopts an individualized, whole-brain connectome approach to examine functional connectivity related to loneliness, characterizing connectivity at three levels: (1) in terms of the pairwise interactions, (2) the network-level interactions, and (3) the global-level topological features. Our individualized connectome-based approach provided broader insight into how selective cognitive changes that characterize loneliness are reflected in the brain's functional organization. Similar to past work examining differences in RSFC related to loneliness (Layden et al., 2017), we found that loneliness was characterized by altered connectivity involving the attention and frontoparietal control networks.

Nevertheless, our connectome approach allowed us to capture other aspects of connectivity associated with loneliness that had not previously been reported. In Chapter 3, these included finding a wide range of changes in connectivity between perceptual and attention networks with frontoparietal and default networks. Crucially, we found that loneliness was associated with lower modular organization between default and frontoparietal networks with externally-directed networks. These results suggest a reduction in network specificity needed to support different aspects of cognition and behavior (e.g., (Petersen & Sporns, 2015; Wig, 2017). In Chapter 4, diverging connectivity patterns associated with loneliness and empathic functioning also open up a novel line of research that could explore whether or how different social contexts influence brain network dynamics. Therefore, although some of our results are consistent with the current neural account of loneliness, they also point to a need to refine the theoretical models of the effects of loneliness on human social cognition.

5.2 Future directions

While the current thesis contributed novel findings to the research on the neural correlates of loneliness, there are many questions that still need to be address and theoretical predictions to be test in future studies. Based on the findings reported here, below are suggestions for several priorities for future research.

5.2.1 Integrating age into the neural account of loneliness

Age is a critical factor influencing social and brain function, yet most neuroimaging studies on loneliness focus on young adult populations. In Chapter 4, we report that that lonely younger and older adults show diverging RSFC patterns, which may, in turn, have implications on empathic functioning. We suggest that the age differences in brain network RSFC related to loneliness may reflect a shift from externally-direct processes in young adults, towards upregulation of brain networks associated with internally-directed cognitive processes in older adults (e.g., mentalizing, perspective-taking, imagining). This shift may begin in middle adulthood through increased connectivity involving the default network (Spreng et al., 2020), and then gradually expand to encompass other higher-order association networks. Critically, the connectivity pattern characterizing loneliness in older adults *does not* align with the ETL model of hypervigilance proposed by Cacioppo et al. (2009). If loneliness in older adults is not driving attentional focus externally, what may be the reason for such a shift?

Considering aging in the experience of loneliness can help foster a more comprehensive developmental account of loneliness as individuals transition from early adulthood to late adulthood. Doing so will require drawing from studies and theories from the cognitive aging literature to explore aspects related to neurocognitive aging and account for how changes in socio-emotional goals contribute to the experience of loneliness, thereby providing a more accurate characterization of the impact of loneliness on brain function. Given the socioemotional shift towards prioritizing meaningful and emotionally gratify-

ing relationships with age (Carstensen, 1992), lonely older adults may engage in more internally-directed mental processes. In Chapter 4, we speculate that greater reliance on reminiscing or mentalizing about future social interactions in lonely older adults may contribute to the shift towards greater internally-directed networks with age. While the study reported in Chapter 4 was not designed to test predictions about the role of mentalizing in loneliness directly, there is evidence that neural representation of attachment figures (mentalizing about a parent or child) is associated with greater activation of cortical midline structures of the default and in the limbic network when perceived closeness to the attachment figures is low (Laurita et al., 2019). The neural representation of familial attachment bonds described by Laurita et al. (2019) exemplifies a pattern of activation that closely matches the network connectivity pattern observed in lonely older adults, where we showed greater connectivity between the default and limbic.

Implementing a network-based approach across multiple modalities of neuroimaging data

The studies included in this thesis focused specifically on functional connectivity. However, anatomical changes associated with loneliness have also been reported (e.g., (Kanai et al., 2012). Extending this network approach to examine structural connectivity represents an important next step in the field as the network functional dynamics of the brain are, in part, constrained by anatomical structure (Honey et al., 2010; Suárez et al., 2020). Research integrating both functional and structural connectivity related to loneliness could enhance our understanding of age-related differences in the functional connectivity patterns observed in the current study. Future work would also benefit from incorporating task-based fMRI approaches. In Chapters 3 and 4, we used resting-state fMRI data to generate the individualized connectomes used to examine brain network connectivity patterns. Prior work has demonstrated that there is a larger gap in how the default network represent the self and others in lonely individuals compared to non-lonely individuals (Courtney & Meyer, 2020). Further, task-based studies may be more

sensitive in predicting brain-behavior relationships when compared to resting-state functional connectivity (Finn & Bandettini, 2021). Future work should test whether default network connectivity in lonely and non-lonely individuals differs between young and older adults. Relatedly, future work should examine the relationship between loneliness and social exclusion/rejection. Using the Cyberball paradigm described in Chapter 2, may be particularly useful for exploring differences in network dynamics related to state and trait loneliness. For example, brain reactivity to social exclusion in lonely and non-lonely individuals could be used to determine whether there is something inherently different at the neural level between state and trait loneliness.

5.2.2 Implications for health and well-being

Because loneliness has been associated with poor mental and physical health outcomes (d'Oleire Uquillas et al., 2018; Ong et al., 2016; Shankar et al., 2013; Wilson et al., 2007), studies that advance our understanding of the impact of loneliness on brain function will have important implications for addressing public health concerns related to loneliness and social isolation, and this may be particularly important in older adulthood, where the implications of loneliness may be even more pronounced. The work presented in Chapter 4 underscores this point. The brain is differentially impacted by loneliness in younger and older adulthood. However, this study was cross-sectional and only measured loneliness at one timepoint. To further test that loneliness related changes in network functional connectivity, future work will need to examine whether the trajectory of these changes in brain functional connectivity change over time by using longitudinal study designs. The older adult participants included in our studies were normally aging, without any clinical indications of non-normative cognitive decline. It would be beneficial to implement this connectome-based approach to compare healthy versus clinical populations and determine whether the patterns of connectivity characterizing loneliness influences the trajectory of neurocognitive aging. Work in our lab has begun exploring these questions (Spreng &

Bzdok, 2021). Additional research looking at other health outcomes such resilience in response to different stressors.

Interventions aimed at reducing loneliness (e.g., improving social skills or promoting social connections) have not proved efficacious (Poscia et al., 2018), especially among older adults. A key limitation to the success of such efforts is that loneliness is a complex and multidimensional construct. A better understanding of the behavioral and neural relationship between loneliness and factors that promote or facilitate prosociality is critical to inform more effective strategies. Chapter 3 considers meaning in life, which is a strong predictor of well-being and inversely related to loneliness. We found that the pattern of connectivity associated with high meaning in life was negatively correlated with loneliness at the behavioral and neural level. Meaning in life may be a relatively modifiable factor (Macia et al., 2021), feelings of loneliness can be attenuated by implementing interventions to encourage introspection and foster a sense of purpose. Finally, throughout this thesis, I stress that the experience of loneliness is specific to the individual. Future studies should would benefit from taking a targeted approach tailored to the specific determinant of loneliness of each individual. Part of the heterogeneity in loneliness is due to factors that contribute to diversity among people (e.g., race, ethnic background, sexual orientation, socioeconomic status). However, this diversity is not reflected within the literature on social and cognitive neuroscience, and this undoubtedly influences the neural and behavioral conclusions drawn from these studies. Future work on loneliness would greatly benefit from the inclusion of underrepresented groups because loneliness is a universal human experience and to ensure that findings on the neural correlates of loneliness are more generalizable to the broader population.

5.3 Conclusion

The impact of loneliness on brain function has only recently been the subject of empirical study. Although much work remains to improve our understanding of the neural basis of

loneliness, the current neural account of loneliness offers a restrictive scope of processes that are inherently variable across people. By utilizing network neuroscience methods, this thesis provides a novel perspective that challenges this one size fits all account. Our findings characterizing individual and age differences in brain network interactions in younger and older adults underscore the need for a more flexible framework for understanding the neural basis of state and trait loneliness throughout adult life. Each of the studies included in this thesis investigates questions about how the experience of loneliness impacts brain network interactions and factors that influence our sense of connection with the external social world at different stages of adulthood. These studies offer a novel view of the functional organizational features that characterize the neural processes underlying state and trait loneliness. In addition, this work expands on previous neuroimaging studies on loneliness by including an older adult cohort and exploring age-related social cognitive changes that have important implications for social functioning in later life.

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