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ORIGINAL ARTICLE

Loneliness in late-life depression: structural and functional connectivity during affective processing

N. M. L. Wong[1,2,3], H.-L. Liu[4], C. Lin[5], C.-M. Huang[6], Y.-Y. Wai[7], S.-H. Lee[8,9]* and T. M. C. Lee[1,2,3,10]*

[1] *Laboratory of Neuropsychology, The University of Hong Kong, Hong Kong*
[2] *Laboratory of Social Cognitive Affective Neuroscience, The University of Hong Kong, Hong Kong*
[3] *Institute of Clinical Neuropsychology, The University of Hong Kong, Hong Kong*
[4] *Department of Imaging Physics, University of Texas MD Anderson Cancer Center, USA*
[5] *Department of Psychiatry, Chang Gung Memorial Hospital, Keelung City, Taiwan*
[6] *College of Biological Science and Technology, National Chiao Tung University, Taiwan*
[7] *Department of Medical Imaging and Intervention, Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[8] *College of Medicine, Chang Gung University, Taiwan*
[9] *Department of Psychiatry, Linkou Chang Gung Memorial Hospital, Taiwan*
[10] *The State Key Laboratory of Brain and Cognitive Science, The University of Hong Kong, Hong Kong*

Background. Late-life depression (LLD) in the elderly was reported to present with emotion dysregulation accompanied by high perceived loneliness. Previous research has suggested that LLD is a disorder of connectivity and is associated with aberrant network properties. On the other hand, perceived loneliness is found to adversely affect the brain, but little is known about its neurobiological basis in LLD. The current study investigated the relationships between the structural connectivity, functional connectivity during affective processing, and perceived loneliness in LLD.

Method. The current study included 54 participants aged >60 years of whom 31 were diagnosed with LLD. Diffusion tensor imaging (DTI) data and task-based functional magnetic resonance imaging (fMRI) data of an affective processing task were collected. Network-based statistics and graph theory techniques were applied, and the participants' perceived loneliness and depression level were measured. The affective processing task included viewing affective stimuli.

Results. Structurally, a loneliness-related sub-network was identified across all subjects. Functionally, perceived loneliness was related to connectivity differently in LLD than that in controls when they were processing negative stimuli, with aberrant networking in subcortical area.

Conclusions. Perceived loneliness was identified to have a unique role in relation to the negative affective processing in LLD at the functional brain connectional and network levels. The findings increas our understanding of LLD and provide initial evidence of the neurobiological mechanisms of loneliness in LLD. Loneliness might be a potential intervention target in depressive patients.

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Introduction

Depression is a highly prevalent disorder affecting the aging population at a considerable prevalence rate of nearly 15% (Beekman *et al.* 1999) and more than one-third of late-life depression (LLD) patients cannot attain full remission after antidepressant treatments (Nelson *et al.* 2008). From neuroimaging and lesion studies, it is often suggested that the neurobiological mechanisms of LLD are related to dysfunction of brain networks and circuitries in the default mode network (i.e. medial prefrontal, posterior cingulate and temporal regions), the salience network (i.e. insula, anterior cingulate and orbitofrontal regions), the cognitive control network (i.e. dorsal lateral prefrontal and posterior parietal regions) and the corticostriatal network (i.e. dorsal lateral prefrontal, lateral orbitofrontal, striatum and thalamus) (Tadayonnejad & Ajilore, 2014). This was determined by studying the regions' task-based functional activations, the functional interdependence of different brain regions, and the integrity of the structural white-matter tracts. Recently, another approach has been adopted that describes the human brain as a graph based on the data from different magnetic resonance imaging (MRI) modalities. The graphs were generated using predefined brain regions as

* Address for correspondence: T. M. C. Lee, Ph.D., Room 656, Laboratory of Neuropsychology, The Jockey Club Tower, The University of Hong Kong, Pokfulam Road, Hong Kong.
(Email: tmclee@hku.hk) [T. M. C. Lee]
(Email: shlee@cgmh.org.tw) [S. -H. Lee]

nodes, and structural [e.g. T1-weighted or diffusion tensor imaging (DTI)] or functional [e.g. functional MRI (fMRI)] connectivity as edges. Usually, axonal projections are inferred from white-matter integrity or streamlines in DTI data and functional interdependence is inferred from correlations in the fMRI data. Under this framework, brain connectivity can be studied in terms of edge weights using an approach such as Network-based Statistics (NBS), and various graph theory network measures can be calculated to quantify the global and the regional network properties of the brain. The global network properties capture the integrity of the information transfer of the whole brain network, whereas regional network properties capture the organization of information transfer across regions in the brain network. It has also been suggested that graph theory measures may be useful in differentiating depression from normal controls and may have potential as biomarkers, bringing important clinical applications (Gong & He, 2015). For instance, LLD patients have been suggested to have different structural global network properties and structural hyperconnectivity in regions within the default mode network, the salience network, and the regions connecting the two in comparison to their healthy counterparts (Ajilore *et al.* 2014*b*). The structural global network property was also found to be negatively related to depression severity in LLD (Ajilore *et al.* 2014*a*). Compared to controls, LLD patients have different hub-like brain structures, including superior temporal gyrus, middle cingulate and putamen (Lim *et al.* 2013). Remitted LLD was also reported to have disrupted structural network organization (Bai *et al.* 2012). Functionally, no global network differences were found between LLD and similarly aged healthy controls, but LLD has been reported to have more functionally connected posterior medial-pareital regions including the medial posterior cingulate and the supramarginal gyrus (Bohr *et al.* 2012). Conversely, decreased regional functional connectivity in LLD in regions across frontal, temporal and parietal lobes (Yuan *et al.* 2008) and between dorsolateral prefrontal cortex and dorsal anterior cingulate cortex has also been reported (Aizenstein *et al.* 2009). Methodological differences and heterogeneous samples used between studies were possible explanations for the inconsistent findings observed in the functional connectivity pattern in LLD. These functional connectivity findings were based on the resting-state fMRI data when subjects were not engaging in any external tasks. Although, changes in functional connectivity in depressed patients while performing emotion tasks was also observed (Frodl *et al.* 2010), there is still limited work focusing on both alterations of the connectivity patterns and network organization in functional networks during task states, which warrants further investigation for better understanding of the neuropsychological mechanisms of implicated cognitive and affective processing in LLD patients.

A key characteristic of LLD is loneliness, which is the perception of being socially isolated resulting from a mismatch between one's social needs and social relationships (Luo *et al.* 2012). People may not feel lonely even living a solitary life, or conversely, can feel lonely despite having a rich social life. Previous research has identified close associations between perceived loneliness and increased depressive symptoms (Cacioppo *et al.* 2006; Hawkley *et al.* 2009); with a more recent study showing loneliness as a predictor of later increases in depressive symptoms over 1-year intervals (Cacioppo *et al.* 2010). The mechanisms involved are still not clear, but it has been suggested that loneliness impairs self-regulation (Baumeister *et al.* 2005) and is associated with strong negative affect (Cacioppo *et al.* 2006). Lonely adults have poor emotion regulation and are less likely to use positive feelings to alleviate their negative mood (Hawkley *et al.* 2009). Loneliness could adversely affect brain structures and neural processes (Cacioppo *et al.* 2014). The gray-matter volume of the posterior superior temporal sulcus and the white-matter tracts involving the inferior frontal gyrus, the temporo-parietal junction and the anterior insula were found to be associated with loneliness (Kanai *et al.* 2012*a*; Tian *et al.* 2014). In lonelier people, weaker functional activations were observed in the ventral striatum when they were viewing pleasant pictures of people than that of objects, and the same was observed in the temporo-parietal junction when they were viewing unpleasant pictures of people (Cacioppo *et al.* 2008). Functional activations in other regions, including the anterior insula, anterior cingulate, the medial frontal gyrus, the dorsomedial prefrontal, inferior orbitofrontal and ventral prefrontal cortexes were reported to be related to social rejection (Eisenberger *et al.* 2003; Cacioppo *et al.* 2013; Powers *et al.* 2013) which could elicit feelings of loneliness (Leary, 1990).

The aforementioned evidences have shown that loneliness is closely related to depression level and might be a potential intervention target (Masi *et al.* 2011; VanderWeele *et al.* 2011; Perissinotto *et al.* 2012). However, little is known about the linkage between loneliness and the brain and how loneliness can be related to LLD, which is characterized by compromised affective processing. To fill this important research gap, the current study explored how loneliness was associated with the structural and functional brain connectivity and network in LLD. NBS, an unbiased connectome-wide approach, was employed to investigate connectional strength and to examine whether there was any connection linking brain

regions that was related to loneliness. Differences between LLD and healthy elderly subjects were then examined in terms of the graph theory network properties [i.e. Small-Worldness (SW), nodal strength and betweenness centrality] within the loneliness-related sub-network identified in NBS. SW characterizes a network that is highly clustered with short average characteristic path length and helps determine whether the brain network has the small-world property. We focused on nodal strength and nodal betweenness centrality that describe the overall connectional strength directly connected to the region and the proportion of shortest paths traversing the region respectively, as both provide good indications of the hub-like feature of the region in the brain. We also investigated how loneliness was associated with affective processing in LLD by capturing the blood oxygen-level dependent signals during a task-based fMRI paradigm of viewing affective stimuli. NBS and graph theory analyses were also applied to the task-based fMRI data similar to the DTI data to characterize the functional connectivity and network properties of LLD when they were processing different types of affective stimuli (i.e. Positive, Negative, or Neutral).

In previous research, structural hyperconnectivity was identified in regions within the default mode network and the salience network in LLD (Ajilore *et al.* 2014*b*). Gray-matter volumetric reduction and disruption of white-matter integrity were detected in LLD within the salience network (Ballmaier *et al.* 2004; Egger *et al.* 2008; Steffens *et al.* 2011) that was important for affective processing and mediation of motivated behaviors (Lindquist *et al.* 2010). It was also reported that aberrant connectivity of the network was related to number of episodes in depression (Meng *et al.* 2014). Functionally, the connectivity and network patterns were less conclusive in LLD patients where both increased and decreased functional connectivity have been reported in different studies (Yuan *et al.* 2008; Aizenstein *et al.* 2009; Bohr *et al.* 2012). We speculated that perceived loneliness, with an adverse effect on the brain, could be associated with the aberrant connectivity and networking prominent in LLD. Therefore, we hypothesized that (1) perceived loneliness would be associated with structural connections within the salience network and that structural hyperconnectivity would exist in this loneliness-related sub-network in LLD. Additionally, negative emotion is associated with alteration of functional activity in areas within salience and corticostriatal networks as proposed by the limbic-cortical model of depression (Mayberg, 1997). Regions within the two networks were also consistently recruited in social rejection scenarios (Cacioppo *et al.* 2013). Therefore, we also hypothesized that (2) perceived loneliness would also be related to functional connectivity in the salience and corticostriatal networks differently when LLD patients were viewing negative stimuli.

Materials and method

Participants

A total of 54 participants aged >60 years was included in the current study. Of these, 31 were classified into the LLD group by two geriatric psychiatrists based on the diagnostic interview of unipolar major depressive disorder according to the Structural Clinical Interview of Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5; APA, 2013). These LLD participants were recruited from a psychiatric out-patient clinic of the Chang Gung Memorial Hospital and experienced their first lifetime major depressive episode at age >50 years. Antidepressants were maintained during the scan due to ethical reasons but their medications in use had not changed at least for 2 weeks prior the scan. Their duration of use of hypnotics was also recorded. Healthy and age-matched elderly controls were recruited by advertisement. All participants were right-handed, as evaluated by the Edinburgh Inventory for Handedness (Oldfield, 1971), and had normal or corrected-to-normal vision. Participants were included only if they did not have any other histories of major psychiatric disorders or cognitive impairment. Additionally, those with a score <24 in the Mini-Mental State Examination (Folstein *et al.* 1975) and a history of other neurological diseases were excluded to ensure no confounding effect from cognitive impairment or other neurological changes.

This study was approved by the Institutional Review Board in the Chang Gung Memorial Hospital, and informed consent was obtained from all participants.

Psychological measures

The 17-item Hamilton Rating Scale for Depression (HAMD) was used to measure the severity of depression in the subjects (Hamilton, 1960). The HAMD has been used widely in psychological and neuropsychiatric studies as an indicator of depressive symptoms and severity of depression, and it has a very high inter-rater reliability on both single items and the total score (Müller & Dragicevic, 2003). It has also been administered to late-life depressed samples in previous MRI studies (Guo *et al.* 2014).

The 20-item UCLA-Loneliness Scale (LS) was used to measure the perceived loneliness of the subjects (Russell, 1996). It was one of the popular measures that was sensitive to perceived loneliness and was distinct from the objective measure of social isolation with

its application extended to older adults and the elderly (Cacioppo *et al.* 2010; Hawkley & Cacioppo, 2010). It has also been used in studying the relationship between loneliness and depressive symptoms (Cacioppo *et al.* 2006).

MRI data acquisition and preprocessing

DTI data, task-based fMRI data, and T1-weighted structural images were acquired on a 3.0 T GE 750 scanner (GE Healthcare Systems, USA) using an 8-channel head coil.

DTI data was collected in 32 diffusion gradient directions ($b = 1000$) with two non-diffusion-weighted ($b = 0$) references using the following parameters: TR = 7500 ms, TE = 80 ms, flip angle = 90°, FOV = 220 × 220 mm^2, voxel size = 1.7 × 1.7 × 2.2 mm^3. Task-based fMRI data was collected with the following parameters: TR = 2000 ms, TE = 30 ms, flip angle = 90°, FOV = 220 × 220 mm^2, voxel size = 3.44 × 3.44 × 4 mm^3. T1-weighted structural imaging data was collected with: TR = 8 ms, TE = 3 ms, FOV = 250 × 250 mm^2, voxel size = 0.98 × 0.98 × 1 mm^3. The T1-weighted images were used for registration during the preprocessing of the fMRI data.

DTI: preprocessing and quantification of structural brain connectivity

The DTI data for each subject was first corrected for eddy current using FSL (Jenkinson *et al.* 2012). Subsequently, the data was fitted with the tensor model, and the streamline tractography algorithm was then applied with whole-brain seeding using DSI Studio (Yeh *et al.* 2013) based on the following parameters and other default settings: number of reconstructed streamlines = 100 000, turning angle = 60°, fractional anisotropy >0.1125, step size = 0.4297, minimum length of streamlines = 10, and maximum length of streamlines = 500.

The Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer *et al.* 2002) comprising 90 cerebral regions was nonlinearly registered to the DTI native space and each region represents a node of the brain network. Structural connection (i.e. edge), with its weight between any pairs of nodal regions, was described by the number of reconstructed streamlines passing the two regions, forming individual raw association matrices denoting the individual's structural brain network.

fMRI: task-based paradigm

The functional brain activations of 47 subjects were measured while they were performing the emotion-processing task (EPT; Lee *et al.* 2012) in the scanner. The EPT is an affective processing task where participants passively viewed 20 positive, 20 negative, and 20 neutral human and non-human pictures from the International Affective Picture System. The pictures used had the highest valence and arousal ratings in published norms during MRI scanning (Bradley & Lang, 2007). All the pictures appeared randomly once on a dark background for 3 s in a 60-trial run, with trials separated by varying durations (i.e. 500, 1000, 1500, 2000 or 2500 ms). The ratings of valence from 1 (very negative) to 9 (very positive) and arousal from 1 (not arousing) to 9 (very arousing) of each picture were collected from each subject off-scanner.

fMRI: preprocessing and functional activations

The fMRI data was preprocessed using FEAT in FSL (Beckmann *et al.* 2006). Each individual's fMRI data was first corrected for motion artifact (i.e. referencing to the middle volume), spatially smoothed (full width at half maximum = 8 mm), high pass temporal filtered (128s), skull-stripped and pre-whitened. The fMRI data of each subject was then normalized to the Montreal Neurological Institute (MNI) standard space through registering to the patient's own skull-stripped T1-weighted volume.

The preprocessed fMRI data was first fed into first level single-subject analyses. The onset and duration of each trial were convolved with the double-gamma hemodynamic response function to approximate the neural event associated with oxy- and deoxy-hemoglobin changes in the blood flow. A general linear model approach was used and three regressors and their temporal derivatives were derived from the positive, negative and neutral pictures. Individuals' functional activations were investigated in the following two contrast conditions: *Positive minus Neutral*, and *Negative minus Neutral*. The results from the two contrast conditions were then brought to the second level group analyses by independent *t* contrasts using mixed effects high-level modeling (i.e. FLAME 1 of FEAT in FSL).

fMRI: quantification of functional brain connectivity

The beta-series correlation approach was employed for which its validity for studying functional connectivity has already been confirmed (Rissman *et al.* 2004). The preprocessed fMRI data was fed again into first-level single-subject analyses using FEAT in FSL. In this approach, a separate regressor was created for each trial of each task condition (i.e. 20 regressors for Positive, 20 regressors for Negative, 20 regressors for Neutral) and was convolved with the double-gamma hemodynamic response function. Using the general linear model, the parameter estimate, or beta, for each trial

at each voxel was obtained and all of them were then sorted by task conditions into beta-series. The AAL atlas (Tzourio-Mazoyer *et al.* 2002) comprising 90 cerebral regions was then registered to the fMRI native space via the corresponding skull-stripped T1-weighted volume and each region represents a node of the functional brain network. Mean parameter estimate was extracted for each trial for each nodal region. Functional connection (i.e. edge), with its weight between any pairs of nodal regions, was then described by the Pearson's correlation between the beta-series of the two regions separately for each task condition. Only edges that were significant after Bonferroni correction were kept to avoid spurious correlations, yielding individual raw association matrices denoting the individual's functional brain network per task condition.

Statistical analysis

Group characteristics and emotion-processing task ratings

Student's t tests were used to compare the characteristics and the psychological measures, and the χ^2 test was used to compare the male-to-female ratio between the LLD group and the healthy elderly. Repeated-measures analysis of variance (rmANOVA) was employed to examine their arousal and valence ratings of affective stimuli.

Task-based functional activations

Moderation of group on the relationship between loneliness and task-based functional activations was analyzed by t contrasts. Group differences in functional activations were also explored. All the t contrasts adopted the voxel-wise threshold at $z > 2.3$ ($p < 0.01$) and cluster extent threshold of $p < 0.05$.

Structural and functional brain connectivity

Similar analytic approach was used on structural and task-based functional raw association matrices. One-sample t tests were first employed separately on individual structural and task-based functional raw association matrices within LLD group and within healthy controls to extract all edges that were significant at 0.05 levels after false-discovery-rate (FDR) corrections. These identified edges within either group were then combined to form a mask. NBS analyses were then performed within the mask to investigate structural or functional connectivity. Using the NBS Connectome toolbox v. 1.2 (Zalesky *et al.* 2010), general linear models were set up and moderation of group on the relationship between loneliness and structural and functional connections was first tested. Specifically for structural connectivity, as no moderation effect was identified, indicating that the association between loneliness and structural connections did not differ between groups, all the subjects were considered together to investigate the relationship between loneliness and structural connectivity across the whole elderly sample with HAMD as covariate regressor. Group differences of structural and functional connectivity between LLD group and healthy controls were also explored. An initial T threshold equivalent to $p < 0.05$ uncorrected was used in the univariate t statistics computed for each edge to identify a set of supra-threshold edges. Non-parametric permutation approach with 1000 permutations was then used to identify the structural or functional connections within the set of supra-threshold edges that survived the cluster extent threshold at $p < 0.05$.

Structural and functional brain network

To further investigate whether the relationships of loneliness with structural and functional connectivity were related to differences in topological organization of structural and functional brain in LLD, graph theory approach was then employed to retrieve graph theory measures for individual's structural and functional brain network of each task condition. A wide range of density thresholds (i.e. $0.0 \leqslant \text{density} \leqslant 0.6$ in steps of 0.01) were first applied on all individual structural and functional matrices using the Matlab-based package GAT (Hosseini *et al.* 2012). At each network density, weighted association matrix was obtained for each subject, and the corresponding graph theory measures, including SW, nodal strength and nodal betweenness centrality were retrieved (Rubinov & Sporns, 2010). For a network G with N nodes and K edges at a particular density, SW was described as the ratio between the normalized clustering coefficient and the normalized characteristic path length, with SW numerically larger than 1 denoting a network having small-world property:

$$SW = \left(\frac{C/C_R}{L/L_R}\right),$$

where C is the cluster coefficient of network G and C_R is the mean clustering coefficient of 100 random networks having the same number of nodes, edges and degree distributions as network G. L is the characteristic path length of network G and L_R is the mean characteristic path length of 100 random networks having the same number of nodes, edges and degree distributions as network G. Nodal strength s_i of each node i was defined as:

$$s_i = \sum_{i \neq j \in N} w_{ij},$$

Table 1. *Group characteristics of LLD subjects and controls*

Group characteristics	LLD ($n = 31$)	Controls ($n = 23$)	Statistical analysis
Age (years)	67.45 (5.427)	67.13 (4.789)	$t_{52} = 0.23$, $p =$ N.S.
Gender (M:F)	14:17	9:14	$\chi^2 = 0.196$, $p =$ N.S.
MMSE	27.84 (1.594)	27.87 (1.632)	$t_{52} = -0.07$, $p =$ N.S.
UCLA-LS	39.97 (11.080)	33.17 (8.316)	$t_{52} = 2.47$, $p = 0.02$
HAMD	11.42 (6.766)	5.65 (4.130)	$t_{52} = 3.61$, $p < 0.001$
Onset age (years)	61.16 (5.826)	–	–
Number of episodes	1.65 (1.380)	–	–
Actual depressed (months)	63.16 (117.122)	–	–
Use of hypnotics (months)	51.84 (42.102)	–	–

LLD, Late-life depression; MMSE, Mini-Mental State Examination; UCLA-LS, UCLA Loneliness Scale; HAMD, Hamilton Rating Scale for Depression; N.S., not significant.

Student's t test or χ^2 test were used.

The results are expressed as the means (standard deviations);

where w_{ij} is the edge weight (i.e. number of streamlines or Pearson's correlation) between nodes i and j. Nodal betweenness centrality b_i of each node i was defined as:

$$b_i = \frac{1}{(n-1)(n-2)} \sum_{\substack{h,j \in N \\ h \neq i, h \neq j, i \neq j}} \frac{\rho^i_{hj}}{\rho_{hj}},$$

where n is the number of nodes (i.e. 90), ρ_{hj} is the number of shortest paths between nodes h and j, and ρ^i_{hj} is the number of shortest paths between nodes h and j passing through node i. A minimum density threshold (i.e. 0.23) was then chosen so that all structural networks were not fragmented, and a maximum density (i.e. 0.30) was chosen based on the SW to avoid random networks (i.e. not random when SW > 1) that are less likely to represent a biologically valid network (Kaiser & Hilgetag, 2006). This maximum density was also chosen in accordance with the depression literature (Korgaonkar *et al.* 2014). The area under the curve (AUC; Ginestet *et al.* 2011) of the graph theory measures (i.e. nodal strength and nodal betweenness centrality) within the defined threshold density range (i.e. 0.23~0.30) of each individual's structural and task-based functional association matrices were then calculated for further analyses on network topological organization. Moderation of group on the relationship between loneliness and AUC of graph theory measures of structural and functional brain networks was analyzed. For structural networks, group differences were compared by Student's t tests in nodal regions that have structural connections related to loneliness. For functional networks, group differences were compared by Student's t tests in nodal regions with functional connections that have different associations with loneliness between LLD and controls. All the calculations were performed using Matlab-based functions implemented in the Brain Connectivity Toolbox (http://brain-connectivity-toolbox.net/) and statistical analyses were carried out in the Matlab environment. The significance of the analyses on graph theory measures was determined at 0.05 levels with FDR corrections.

Results

Group characteristics

The age, gender ratio and MMSE scores did not differ between groups (all p's > 0.05), confirming that the two groups were matched, and the LLD group had higher scores in the LS ($t_{52} = 2.47$, $p = 0.02$) and the HAMD ($t_{52} = 3.61$, $p < 0.001$) (Table 1).

Loneliness and structural brain

Association between loneliness and structural brain connectivity

From NBS analyses, group did not moderate the relationship between scores in LS and any structural connections. Across all subjects, scores in LS were identified to be negatively associated with edge weights of edges within a sub-network component comprising of 10 nodal regions and 10 edges. This loneliness-related sub-network identified from the whole elderly sample included edges of right middle temporal gyrus, amygdala, fusiform gyrus, inferior occipital gyrus, middle occipital gyrus, superior occipital gyrus, and calcarine fissure connecting to left globus pallidus, and edges from right calcarine fissure, lingual gyrus and amygdala connecting to superior frontal gyrus (Fig. 1).

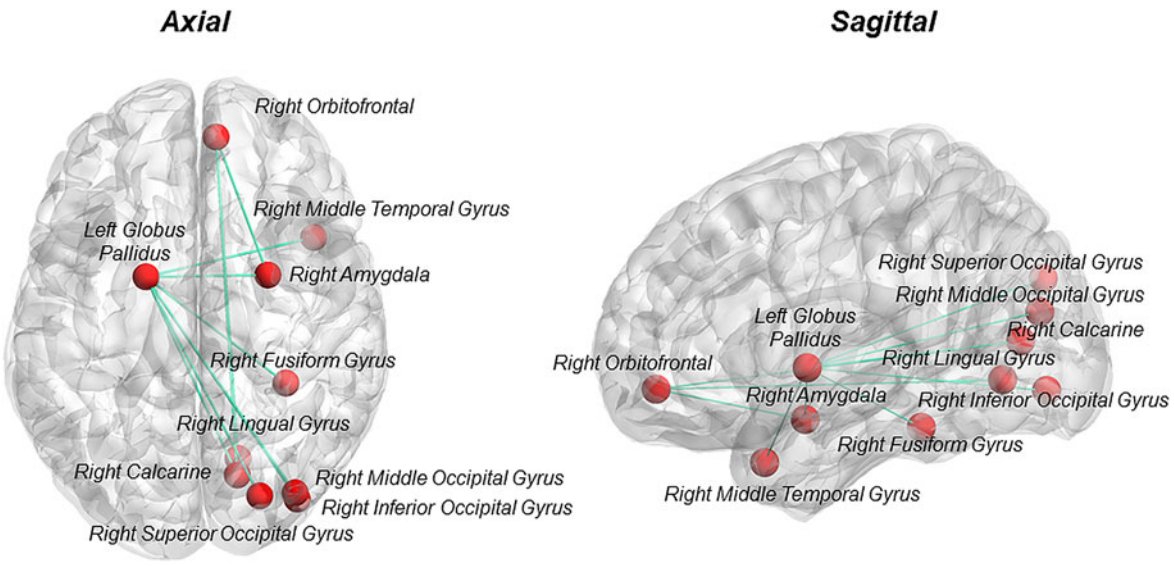

Fig. 1. Summary of significant sub-network that is associated with subjects' scores in UCLA Loneliness Scale using network-based statistical analysis. The nodes and edges of this sub-network are overlaid on the ICBM152 brain surface (http://surfer.nmr.mgh.harvard.edu/) and the axial and sagittal views are shown.

Group comparison in structural brain connectivity and network

From NBS analyses, no significant differences in structural connection between LLD and controls were identified. Using graph theory analyses, the group differences in AUC of nodal degree and betweenness centrality of the 10 regions within the loneliness-related sub-network were then examined by individual Student's t tests. None of the nodal graph theory measures showed any significant differences between LLD and controls after FDR corrections for multiple comparisons (Supplementary Table S1).

Loneliness and functional brain of affective processing

Association between loneliness and arousal and valence ratings

Moderation of group on the relationship between scores in LS and the subjects' arousal and valence ratings of the emotional stimuli (i.e. Positive, Negative, Neutral) did not yield any significant results (all p's > 0.05). Therefore, partial Pearson's correlations between scores in LS and all subjects' arousal and valence ratings were performed, controlling for HAMD. It was found that all the arousal ratings negatively correlated with scores in LS (Positive: $r_{44} = -0.33$, $p = 0.02$; Negative: $r_{44} = -0.33$, $p = 0.03$; Neutral: $r_{44} = -0.42$, $p = 0.004$). The correlations were similar after further controlling for group.

Association between loneliness and functional activations during affective processing

Significant moderation of group on the relationship between scores in LS and the subjects' functional activations was observed in contrast condition *Negative minus Neutral*, forming a large cluster comprising mainly insula, putamen, globus pallidus and brain stem, along with frontal regions including inferior frontal gyrus, frontal pole, and frontal orbital cortex ($k = 4670$, $Z_{peak} = 3.69$, MNI coordinates of Z_{peak}: −24, −4, −4) (Fig. 2*a*), of which the percent signal change negatively correlated with scores in LS in LLD ($r_{25} = -0.39$, $p = 0.04$) but positively correlated with that in controls ($r_{18} = 0.52$, $p = 0.02$). Similar findings were observed after controlling for HAMD in partial Pearson's correlation. No moderation was observed in contrast condition *Positive minus Neutral*.

Association between loneliness and functional connectivity during affective processing

From NBS analyses, significant moderation of group on the relationship between scores in LS and the subjects' functional connectivity was observed in connections linking a widespread of regions in Negative and Neutral conditions. Therefore, a more stringent T threshold ($p < 0.01$) with cluster extent threshold at $p < 0.05$ was applied and reported. For the Negative condition, 33 nodes within a sub-network component of 46 edges were related to scores in LS positively in LLD but negatively in controls, mainly involving frontal regions, along with posterior part of cingulum, thalamus, and some temporal and occipital areas (Table 2, Fig. 2*b*). For the Neutral condition, 67 nodes within a sub-network component of 132 edges were related to scores in LS more positively in controls than that in LLD (Supplementary Table S2). No moderation effect

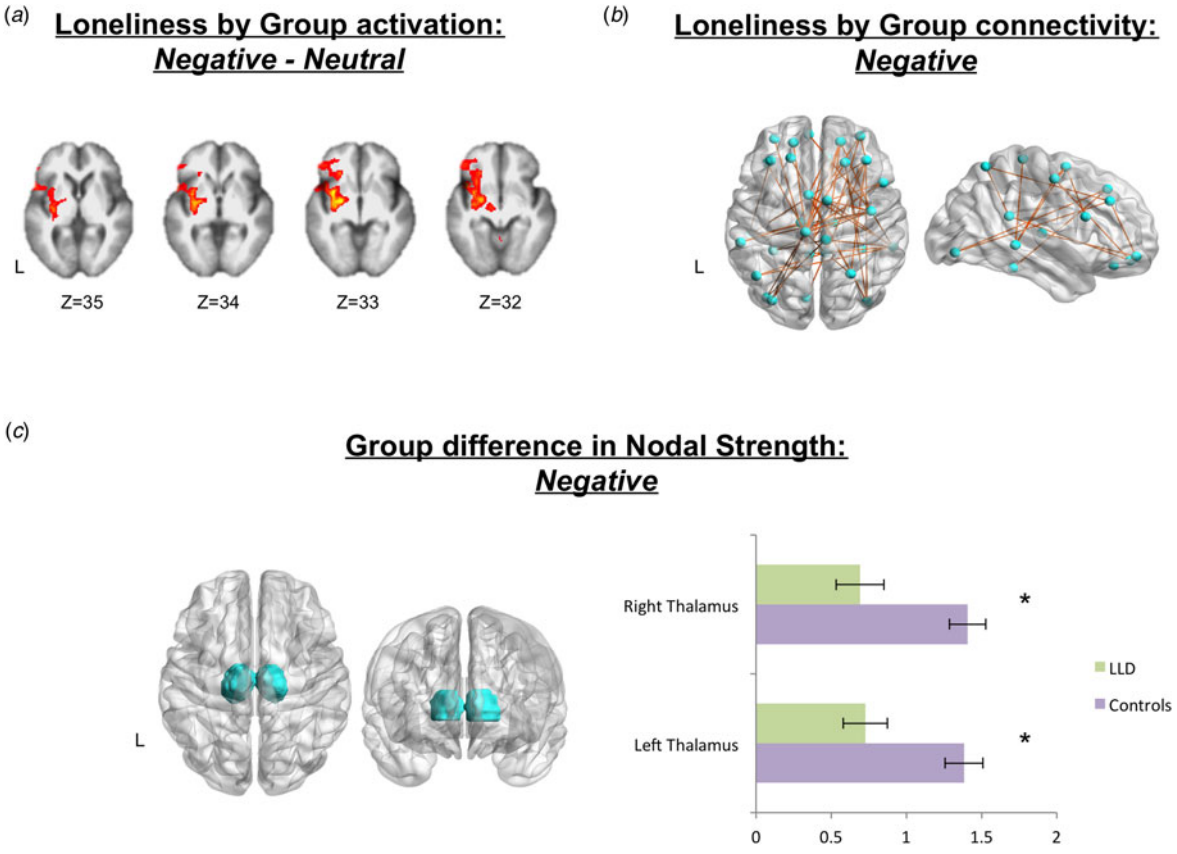

Fig. 2. (*a*) The functional clusters having significantly different associations with scores in Loneliness scale (LS)between late-life depression (LLD) and controls in the Negative minus Neutral contrast condition are overlaid in red-yellow on the corresponding template in standard Montreal Neurological Institute space. (*b*) The functional nodes and edges having significantly different associations with scores in LS between LLD and controls during Negative condition are overlaid on the ICBM152 brain surface (http://surfer.nmr.mgh.harvard.edu/) and the axial and sagittal views are shown. (*c*) The regions of interest, both left and right thalamus, are overlaid on the ICBM152 brain surface (http://surfer.nmr.mgh.harvard.edu/) with the corresponding bar charts with standard error showing the significant group differences in the area under the curve of nodal strength in the two regions. L, Left.

Table 2. *Pearson's correlation between functional connections of nodal pairs and scores in UCLA-Loneliness Scale in LLD subjects and controls during Negative task condition*

		Pearson's *R*	
Nodal region *i*	Nodal region *j*	LLD ($n = 27$)	Controls ($n = 20$)
Precentral_R	Frontal_Sup_Orb_R	0.46	−0.43
Frontal_Mid_R	Frontal_Mid_Orb_R	0.34	−0.44
Precentral_R	Supp_Motor_Area_R	0.45	−0.48
Frontal_Inf_Oper_R	Supp_Motor_Area_R	0.65	−0.25
Precentral_R	Frontal_Med_Orb_L	0.49	−0.42
Supp_Motor_Area_L	Rectus_R	0.36	−0.44
Frontal_Sup_Orb_L	Cingulum_Post_L	0.16	−0.47
Frontal_Sup_Orb_R	Cingulum_Post_L	0.26	−0.47
Frontal_Mid_Orb_L	Cingulum_Post_L	0	−0.47
Frontal_Sup_Orb_R	Cingulum_Post_R	0.20	−0.47
Frontal_Mid_R	Calcarine_L	0.26	−0.49
Frontal_Mid_R	Occipital_Mid_L	0.41	−0.50

Table 2 (*cont.*)

Nodal region *i*	Nodal region *j*	Pearson's *R*	
		LLD ($n = 27$)	Controls ($n = 20$)
Frontal_Sup_R	Occipital_Inf_L	0.46	−0.48
Frontal_Sup_L	Occipital_Inf_R	0.36	−0.52
Frontal_Sup_R	Occipital_Inf_R	0.36	−0.41
Frontal_Mid_R	Occipital_Inf_R	0.39	−0.40
Supp_Motor_Area_L	Occipital_Inf_R	0.51	−0.42
Precentral_R	Fusiform_L	0.44	−0.43
Frontal_Sup_R	Fusiform_R	0.27	−0.44
Frontal_Mid_R	Parietal_Sup_R	0.53	−0.35
Cingulum_Post_R	Parietal_Sup_R	0.25	−0.46
Cingulum_Post_R	Angular_L	0.38	−0.43
Precentral_R	Paracentral_Lobule_L	0.54	−0.44
Frontal_Sup_R	Paracentral_Lobule_L	0.52	−0.39
Frontal_Sup_Orb_R	Paracentral_Lobule_L	0.32	−0.47
Frontal_Inf_Oper_R	Paracentral_Lobule_L	0.53	−0.34
Frontal_Inf_Orb_L	Paracentral_Lobule_L	0.43	−0.43
Rectus_R	Paracentral_Lobule_L	0.39	−0.44
Cingulum_Post_R	Paracentral_Lobule_L	0.31	−0.47
Occipital_Mid_L	Paracentral_Lobule_L	0.51	−0.39
Occipital_Inf_L	Paracentral_Lobule_L	0.49	−0.38
Occipital_Inf_L	Paracentral_Lobule_R	0.49	−0.38
Fusiform_L	Paracentral_Lobule_R	0.34	−0.47
Frontal_Mid_Orb_R	Thalamus_L	0.16	−0.46
SupraMarginal_L	Thalamus_L	0.21	−0.48
Frontal_Mid_Orb_R	Thalamus_R	0.16	−0.47
Precentral_R	Temporal_Mid_L	0.30	−0.44
Precentral_R	Temporal_Mid_R	0.47	−0.44
Supp_Motor_Area_L	Temporal_Mid_R	0.34	−0.44
Supp_Motor_Area_R	Temporal_Mid_R	0.42	−0.44
Cingulum_Post_L	Temporal_Mid_R	0.27	−0.47
Angular_L	Temporal_Mid_R	0.20	−0.48
Paracentral_Lobule_R	Temporal_Mid_R	0.31	−0.47
Precentral_R	Temporal_Inf_L	0.36	−0.43
Frontal_Mid_L	Temporal_Inf_L	0	−0.44
Frontal_Mid_R	Temporal_Inf_L	0	−0.45

The Pearson's *R* of the connections between any two nodes (*i, j*) that were identified to have significant Loneliness by Group interaction are reported for LLD and controls.

LLD, Late-life depression; Precentral_R, right precentral gyrus; Frontal_Sup_L, left superior frontal gyrus, dorsolateral; Frontal_Sup_R, right superior frontal gyrus, dorsolateral; Frontal_Sup_Orb_L, left superior frontal gyrus, orbital part; Frontal_Sup_Orb_R, right superior frontal gyrus, orbital part; Frontal_Mid_L, left middle frontal gyrus, lateral part; Frontal_Mid_R, right middle frontal gyrus, lateral part; Frontal_Mid_Orb_L, left middle frontal gyrus, orbital part; Frontal_Mid_Orb_R, right middle frontal gyrus, orbital part; Frontal_Inf_Oper_R, right opercular part of inferior frontal gyrus; Frontal_Inf_Orb_L, left orbital part of inferior frontal gyrus; Supp_Motor_Area_L, left supplementary motor area; Supp_Motor_Area_R, right supplementary motor area; Frontal_Med_Orb_L, left superior frontal gyrus, medial orbital part; Rectus_R, right gyrus rectus; Cingulum_Post_L, left posterior cingulate gyrus; Cingulum_Post_R, right posterior cingulate gyrus; Calcarine_L, left calcarine sulcus; Occipital_Mid_L, left middle occipital; Occipital_Inf_L, left inferior occipital; Occipital_Inf_R, right inferior occipital; Fusiform_L, left fusiform gyrus; Fusiform_R, right fusiform gyrus; Parietal_Sup_R, right superior parietal lobule; SupraMarginal_L, left supramarginal gyrus; Angular_L, left angular gyrus; Paracentral_Lobule_L, left paracentral lobule; Paracentral_Lobule_R, right paracentral lobule; Thalamus_L, left thalamus; Thalamus_R, right thalamus; Temporal_Mid_L, left middle temporal gyrus; Temporal_Mid_R, right middle temporal gyrus; Temporal_Inf_L, left inferior temporal gyrus.

Table 3. *Emotion-processing task ratings from late-life depression (LLD) subjects and controls*

Ratings	LLD ($n = 27$)	Controls ($n = 20$)
Arousal		
Positive	91.52 (57.045)	77.05 (59.964)
Negative	95.41 (53.733)	94.75 (56.776)
Neutral	53.19 (35.786)	41.90 (24.203)
Valence		
Positive	154.41 (11.547)	150.55 (18.132)
Negative	41.11 (8.794)	40.65 (15.786)
Neutral	108.74 (14.899)	105.25 (9.060)

Means with standard deviations in parentheses are presented.

of group on the relationship between scores in LS and subjects' functional connectivity in Positive condition was identified.

Group comparison in arousal and valence ratings

From the rmANOVA, no significant condition by group interaction was observed in the subjects' valence ratings ($F_{2,90} = 0.24$, $p = 0.79$). There was a significant main effect of condition on valence ratings ($F_{2,90} = 896.41$, $p < 0.001$) but no main effect of group was identified ($F_{1,90} = 1.19$, $p = 0.28$). *Post-hoc* pairwise comparisons with Bonferroni adjustment revealed that valence ratings differed between any two conditions (all p's < 0.001) with the mean ratings in the order of Positive > Neutral > Negative. Similarly, no significant condition by group interaction was observed in their arousal ratings ($F_{2,90} = 0.83$, $p = 0.44$). There was a significant main effect of condition on arousal ratings ($F_{2,90} = 39.52$, $p < 0.001$) but no main effect of group was identified ($F_{1,90} = 0.45$, $p = 0.51$). *Post-hoc* pairwise comparisons with Bonferroni adjustment revealed that arousal ratings differed between any two conditions (all p's < 0.05) with the mean ratings in the order of Negative > Positive > Neutral. The means of task ratings are presented in Table 3.

Group comparison in functional activations of affective processing

The LLD group exhibited larger functional activations in both *Positive minus Neutral* and *Negative minus Neutral* contrast conditions. In the *Positive minus Neutral* contrast, a large cluster comprising mainly frontal pole, paracingulate gyrus, and frontal medial and orbital cortex with some activations in the subcortical regions such as caudate, nucleus accumbens and putamen was observed to be more activated in LLD ($k = 2177$, $Z_{peak} = 3.62$, MNI coordinate of Z_{peak}: −14, 54, −6); while in the *Negative minus Neutral* contrast, a very widespread and large cluster comprising frontal pole, frontal orbital cortex, middle temporal gyrus, precuneus and cingulate regions along with subcortical regions including hippocampus caudate, putamen, globus pallidus, nucleus accumbens, amygdala, and also brain stem was observed to be more activated in LLD as well ($k = 42\,826$, $Z_{peak} = 4.67$, MNI coordinates of Z_{peak}: 60, −56, −26).

Group comparison in functional brain connectivity and network of affective processing

From NBS analyses, the LLD group and controls did not show any differences in the functional connectivity in any of the task conditions. Using graph theory analyses, significant group differences were observed in the nodal strength of thalamus (left thalamus: $t_{45} = 3.39$, $p = 0.02$; right thalamus: $t_{45} = 3.63$, $p = 0.02$) (Fig. 2*c*) that has functional connections associated with scores in LS differently between LLD and controls in Negative condition.

Discussion

The findings partially supported our first hypothesis that perceived loneliness was associated with structural brain networking in regions within the salience network (i.e. amygdala), with an additional component that connected left globus pallidus with temporal and occipital regions. No structural hyperconnectivity within this loneliness-related sub-network was identified. Functionally, connectivity within the default mode network and the corticostriatal network was found to be positively associated with the perceived loneliness in LLD but negatively with that in controls when processing negative stimuli. We further revealed that the thalamus from the corticostriatal network had decreased hub-like networking pattern in LLD. This finding only partially aligned with our initial hypothesis. We believe that verifying the two hypotheses would increase our knowledge of the neurobiological model of LLD at the brain connectivity and networking levels, and importantly, would provide some evidence on how loneliness could be closely related to the disease from a social neuroscientific perspective. To the best of our knowledge, this was the first study investigating how perceived loneliness in LLD was related to their brain connectivity and networking during affective processing.

Loneliness is associated with structural brain networking

Studies investigating the structural brain networking in LLD are scarce. In the current study, we identified

loneliness-related sub network connecting the frontal (i.e. superior frontal gyrus) to the limbic area (i.e. amygdala), and an additional component that connected left globus pallidus with temporal and occipital regions. From the literature, amygdala was found to be not just responsible for affective processing (e.g. Lindquist *et al.* 2010), but it also plays an important role in social cognition and social connectedness (Kanai *et al.* 2012*b*). Strength of functional connectivity connecting from amygdala to frontal regions could predict the social networking size (Bickart *et al.* 2012). There was also research suggesting superior frontal gyrus as one of the regions that was functionally reacted to social exclusion situations (Bolling *et al.* 2011). Perceived loneliness feeling is aroused from the perception of being socially isolated when there is mismatch between one's social needs and the social relationships provided by one's social network (Luo *et al.* 2012). Our finding of higher perceived loneliness related to weaker structural connection between the amygdala and superior frontal gyrus might further imply that the perceived loneliness feeling in elderly would also be associated with structural connections emanating from amygdala. Less direct evidences were reported on the relationship between globus pallidus and/or its connectivity and loneliness. From a recent study, asymmetry of globus pallidus volume has been reported to be related to social avoidance, and that its volume was associated with interpersonal relationships (Evans *et al.* 2015). Functional alterations in globus pallidus and its functional connectivity have been detected in patients with social anxiety disorder (Hattingh *et al.* 2012; Arnold Anteraper *et al.* 2014). The social exclusion theory of anxiety has suggested that anxiety can be elicited by one's fear of being excluded from important social groups (Baumeister & Tice, 1990), as such, affective reactions including social anxiety and the loneliness feeling often exhibit as response (Leary, 1990). Therefore, structural connections from globus pallidus might also be important in responding to social exclusion situations and hence, related to the perceived loneliness.

Our findings have revealed that perceived loneliness did not act on the structural connectivity differently in LLD as compared to controls and no alteration of nodal strength in regions within the loneliness-related sub-network in LLD patients was identified. This did not align with part of our initial hypothesis of expecting structural hyperconnectivity in the salience network in LLD. Methodological differences may be one of the possible explanations. The current study has used DTI data to study structural connectivity whereas structural hyperconnectivity observed in regions within the default mode network and the salience network was previously implied by T1-weighted data (Ajilore *et al.* 2014*b*). It may reveal that there are significant dependencies of structural network measures on methodologies and warrants more careful interpretation of the alterations of structural networking patterns in the LLD patients. Another possible explanation may relate to the different LLD patient sample included in the current study. Antidepressants were maintained in the LLD patients during the scanning because of ethical reasons in the current study. We cannot rule out the possibility that the inconsistent structural networking pattern observed was related to the medication effect. Nonetheless, the current study has proved that higher perceived loneliness in LLD patients and healthy elderly is related to a large extent of brain structural disconnectivity. It suggested that there was a detectable impact of the subjective feeling of loneliness in LLD and elderly at the structural brain connectional level.

Special role of loneliness in the functional connectivity of negative affective processing in LLD

Contrasting to the non-significant moderation effect of group on the relationship between one's perceived loneliness and structural connectivity, significant moderation effect of group on the relationship between one's perceived loneliness and functional activations was observed in contrast condition *Negative minus Neutral* within regions including insula, basal ganglia and orbitofrontal area. Furthermore, the functional connectivity within the default mode network (i.e. middle frontal gyrus, posterior cingulate, middle and inferior temporal gyrus) and the corticostriatal network (i.e. lateral orbitofrontal and thalamus) during negative affective processing was positively associated with perceived loneliness in LLD but negatively associated with that in the healthy elderly. The default mode network is involved in perspective taking and self-reflection processes (Buckner & Carroll, 2007). It activates more when one focuses on more internal and self-referential processes, and deactivates when one focuses on goal-directed tasks or the external environment (Raichle *et al.* 2001) Importantly, failing in down-regulating the default mode network activations when viewing negative stimuli has been reported in depressed patients and was seen as a network-based mechanism of depression (Sheline *et al.* 2009). The functional connectivity between posterior cingulate and middle temporal gyrus is identified to be more responsive to emotion (Laird *et al.* 2009). Furthermore, most of the other subnetworks of default mode network are found to originate in posterior cingulate cortex. Previously, posterior cingulate cortex has been reported to be related to perceived social support (Che *et al.* 2014), and hence, it is intriguing to propose that perceived loneliness plays a special role in LLD

and has an impact on the core anchor of the default mode network which would be related to the failure in the network down-regulation during the processing of negative emotions.

Corticostriatal circuitry, that is densely interconnected among frontal regions, basal ganglia and thalamus, plays an important role in reward processing and hedonic experience; and it was also reported to be abnormal in LLD and could be contributing to the apathy and anhedonia experience in LLD (Tadayonnejad & Ajilore, 2014). LLD patients were reported to have reduced gray matter volume over orbitofrontal cortex, putamen, and thalamus (Sexton *et al.* 2013). From the limbic-cortical model of depression, negative emotions in depressed patients were particularly related to the dysregulation of the functional circuitry, of which the basal ganglion would receive segregated afferents and efferents from both the dorsal (i.e. anterior and posterior cingulate) and ventral (i.e. subgenual, anterior insula, hypothalamus) compartments with thalamic region as a relay (Mayberg, 1997). It was also pointed out by another meta-analytic study that the thalamus closely works with the frontal system to control over other subcortical structures (i.e. striatum) for the primitive responses towards emotions (Lai, 2014). On the other hand, thalamus has been reported to have changes in activations when people encounter social pain situations, such as romantic rejection, bereavement and social rejection scenarios (Eisenberger, 2012). As argued by Keltner & Kring (1998), emotions carry social meanings and are critical in coordinating social interactions. Norris *et al.* (2004) have also conducted a task-based fMRI study to investigate how different regions may react to emotional and social pictures and thalamus was one of the regions that were responsive to emotional content and more strongly with social stimuli. They argued that social stimuli is emotionally evocative that catalyzes emotional reactions, which might echo the current study's findings on how thalamic connectivity, as part of a key circuitry implicated in processing negative emotions in depressed patients, has a different association with their subjective feeling of loneliness. More interestingly, we further identified that the thalamus had decreased hub-like networking pattern in LLD. It is tentatively proposed that alterations in the functional connectivity of the corticostriatal circuitry because of the detrimental effect of being lonely might be related to the less efficient networking of the thalamus as a relay center. Prospective studies are needed for validation. It is noted that no other functional network differences in terms of nodal strength and nodal betweenness centrality were revealed in other regions, which might not align with previous findings (Yuan *et al.* 2008; Aizenstein *et al.* 2009; Bohr *et al.* 2012). As proposed earlier, inconsistent findings observed in the functional connectivity pattern in LLD might be attributed to significant methodological differences between studies. Particularly, the current study is investigating the networking differences between groups during negative affective processing instead of resting-state. Additionally, we have only focused our graph theory analyses on regions that showed different association with perceived loneliness in LLD because the current study aims to investigate the special role of loneliness in LLD at the brain connectivity and network levels, and hence, might have overlooked some of the other potential nodal networking differences.

Finally, it is also worth noting that there is no moderation effect of group on the association between subjects' perceived loneliness and their arousal and valence ratings of the affective stimuli. This might suggest that the impact of perceived loneliness on affective processing in LLD is more sensitive at the brain connectivity and network levels, but less likely to be represented at the behavioral level.

Implications

LLD is related to dysfunction of brain networks, and this association has gained support from putative neuroimaging and lesions studies (Drevets *et al.* 2008). To effectively alleviate depressive symptoms in depressed elderly, it is crucial to identify psychotherapeutic interventional targets that can promote neurobiological changes at the network level. The current study has shown how perceived loneliness is closely related to the brain connectivity and networking. Moreover, we have shown that perceived loneliness plays a unique role in the implicated affective processing in LLD in terms of functional connectivity and networking, whereas the pattern of structural connectivity associated with perceived loneliness appears to be largely similar across both LLD and healthy elderly. Our study provides first evidence indicating that perceived loneliness might be detrimental to brain circuitries that underlie affective processing in LLD. Because not all LLD patients can attain full remission after antidepressant treatments (Nelson *et al.* 2008), targeting their perceived loneliness might be a useful intervention scheme for depressive patients. Previous literature has already shown how loneliness could be managed and remediated practically through providing social support, training on social skills and social cognition (Masi *et al.* 2011; VanderWeele *et al.* 2011; Perissinotto *et al.* 2012). This study has increased our understanding of the neurobiological underpinnings of loneliness and has brought clinical value to treating depressive symptoms.

Limitations

We have provided initial evidence indicating that loneliness could be related to alteration of functional

networking in LLD, but we could not be certain how this was related to the physiology of the brain and the disease. The LLD patients were on antidepressants due to ethical reasons but potential medication effect could have confounded our findings. We have only recruited those with medications in use that have not changed at least for 2 weeks prior the MRI scanning to avoid abrupt neural and physiological changes brought by changing of medications. In addition, the current study could not address the specificity of the findings because only LLD subjects and controls were compared. It would be interesting if additional comparisons were made, for example, between early onset depressed patients and LLD patients or between other clinical populations (e.g. bipolar disorder) and LLD patients. These comparisons could be made to investigate the potential commonalities and specificities of the disease at the brain connectivity and networking levels and the effectiveness of loneliness as an intervention target. Future work along this research direction would be informative.

Conclusions

Perceived loneliness is a crucial variable in the neurobiological model of LLD, relating to both the alteration in functional connectivity and networking in negative affective processing. Targeting at the social connectedness and loneliness feeling in LLD might be worth considered as remediation to LLD. Prospective studies in neuropsychological basis of loneliness and depression are much encouraged.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291716001033.

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Declaration of Interest

None.

References

Aizenstein HJ, Butters MA, Wu M, Mazurkewicz LM, Stenger VA, Gianaros PJ, Becker JT, Reynolds III CF, Carter CS (2009). Altered functioning of the executive control circuit in late-life depression: episodic and persistent phenomena. *American Journal of Geriatric Psychiatry* **17**, 30–42.

Ajilore O, Lamar M, Kumar A (2014*a*). Association of brain network efficiency with aging, depression, and cognition. *American Journal of Geriatric Psychiatry* **22**, 102–110.

Ajilore O, Lamar M, Leow A, Zhang A, Yang S, Kumar A (2014*b*). Graph theory analysis of cortical-subcortical networks in late-life depression. *American Journal of Geriatric Psychiatry* **22**, 195–206.

APA (2013). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. American Psychiatric Association: Washington.

Arnold Anteraper S, Triantafyllou C, Sawyer AT, Hofmann SG, Gabrieli JD, Whitfield-Gabrieli S (2014). Hyper-connectivity of subcortical resting-state networks in social anxiety disorder. *Brain Connectivity* **4**, 81–90.

Bai F, Shu N, Yuan Y, Shi Y, Yu H, Wu D, Wang J, Xia M, He Y, Zhang Z (2012). Topologically convergent and divergent structural connectivity patterns between patients with remitted geriatric depression and amnestic mild cognitive impairment. *Journal of Neuroscience* **32**, 4307–4318.

Ballmaier M, Toga AW, Blanton RE, Sowell ER, Lavretsky H, Peterson J, Pham D, & Kumar A (2004). Anterior cingulate, gyrus rectus, and orbitofrontal abnormalities in elderly depressed patients: an MRI-based parcellation of the prefrontal cortex. *American Journal of Psychiatry* **161**, 99–108.

Baumeister R, Tice D (1990). Point-counterpoints: anxiety and social exclusion. *Journal of Social and Clinical Psychology* **9**, 165–195.

Baumeister RF, DeWall CN, Ciarocco NJ, Twenge JM (2005). Social exclusion impairs self-regulation. *Journal of Personality and Social Psychology* **88**, 589–604.

Beckmann CF, Jenkinson M, Woolrich MW, Behrens TEJ, Flitney DE, Devlin JT, Smith SM (2006). Applying FSL to the FIAC data: model-based and model-free analysis of voice and sentence repetition priming. *Human Brain Mapping* **27**, 380–391.

Beekman ATF, Copeland JRM, Prince MJ (1999). Review of community prevalence of depression in later life. *British Journal of Psychiatry* **174**, 307–311.

Bickart KC, Hollenbeck MC, Barrett LF, Dickerson BC (2012). Intrinsic amygdala-cortical functional connectivity predicts social network size in humans. *Journal of Neuroscience* **32**, 14729–14741.

Bohr IJ, Kenny E, Blamire A, O'Brien JT, Thomas AJ, Richardson J, Kaiser M (2012). Resting-state functional connectivity in late-life depression: higher global connectivity and more long distance connections. *Frontiers in Psychiatry* **3**, 116.

Bolling DZ, Pitskel NB, Deen B, Crowley MJ, Mayes LC, Pelphrey KA (2011). Development of neural systems for processing social exclusion from childhood to adolescence. *Developmental Science* **14**, 1431–1444.

Bradley MM, Lang PJ (2007). The International Affective Picture System (IAPS) in the study of emotion and

attention. In *Handbook of Emotion Elicitation and Assessement* (ed. J. A. Coan and J. J. B. Allen), pp. 29–46. Oxford University Press: Oxford.

Buckner RL, Carroll DC (2007). Self-projection and the brain. *Trends in Cognitive Sciences* **11**, 49–57.

Cacioppo JT, Hawkley LC, Thisted RA (2010). Perceived social isolation makes me sad: 5-year cross-lagged analyses of loneliness and depressive symptomatology in the Chicago Health, Aging, and Social Relations Study. *Psychology and Aging* **25**, 453–463.

Cacioppo JT, Hughes ME, Waite LJ, Hawkley LC, Thisted RA (2006). Loneliness as a specific risk factor for depressive symptoms: cross-sectional and longitudinal analyses. *Psychology and Aging* **21**, 140–151.

Cacioppo JT, Norris CJ, Decety J, Monteleone G, Nusbaum H (2008). In the eye of the beholder : individual differences in perceived social isolation predict regional brain activation to social stimuli. *Journal of Cognitive Neuroscience* **21**, 83–92.

Cacioppo S, Capitanio JP, Cacioppo JT (2014). Toward a neurology of loneliness. *Psychological Bulletin* **140**, 1464–1504.

Cacioppo S, Frum C, Asp E, Weiss RM, Lewis JW, Cacioppo JT (2013). A quantitative meta-analysis of functional imaging studies of social rejection. *Scientific Reports* **3**, 2027.

Che X, Wei D, Li W, Li H, Qiao L, Qiu J, Zhang Q, Liu Y (2014). The correlation between gray matter volume and perceived social support: a voxel-based morphometry study. *Social Neuroscience* **9**, 152–159.

Drevets WC, Price JL, Furey ML (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Structure and Function* **213**, 93–118.

Egger K, Schocke M, Weiss E, Auffinger S, Esterhammer R, Goebel G, Walch T, Mechtcheriakov S, & Marksteiner J (2008). Pattern of brain atrophy in elderly patients with depression revealed by voxel-based morphometry. *Neuroimaging* **164**, 237–244.

Eisenberger NI (2012). The pain of social disconnection: examining the shared neural underpinnings of physical and social pain. *Nature Reviews Neuroscience* **13**, 421–434.

Eisenberger NI, Lieberman MD, Williams KD (2003). Does rejection hurt? An fMRI study of social exclusion. *Science* **302**, 290–292.

Evans DW, Lazar SM, Boomer KB, Mitchel AD, Michael AM, Moore GJ (2015). Social cognition and brain morphology: implications for developmental brain dysfunction. *Brain Imaging and Behavior* **9**, 264–274.

Folstein MF, Folstein SE, McHugh PR (1975). 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research* **12**, 189–198.

Frodl T, Bokde ALW, Scheuerecker J, Lisiecka D, Schoepf V, Hampel H, Möller HJ, Brückmann H, Wiesmann M, Meisenzahl E (2010). Functional connectivity bias of the orbitofrontal cortex in drug-free patients with major depression. *Biological Psychiatry* **67**, 161–167.

Ginestet CE, Nichols TE, Bullmore ET, Simmons A (2011). Brain network analysis: separating cost from topology using cost-integration. *PLoS ONE* **6**, e21570.

Gong Q, He Y (2015). Depression, neuroimaging and connectomics: a selective overview. *Biological Psychiatry* **77**, 223–235.

Guo H, Cheng C, Cao X, Xiang J, Chen J, Zhang K (2014). Resting-state functional connectivity abnormalities in first-onset unmedicated depression. *Neural Regeneration Research* **9**, 153–163.

Hamilton M (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry* **23**, 56–63.

Hattingh CJ, Ipser J, Tromp SA, Syal S, Lochner C, Brooks SJ, Stein DJ (2012). Functional magnetic resonance imaging during emotion recognition in social anxiety disorder: an activation likelihood meta-analysis. *Frontiers in Human Neuroscience* **6**, 347.

Hawkley LC, Cacioppo JT (2010). Loneliness and blood pressure in older adults: defining connections. *Aging Health* **6**, 415–418.

Hawkley LC, Thisted RA, Cacioppo JT (2009). Loneliness predicts reduced physical activity: cross-sectional & longitudinal analyses. *Health Psychology* **28**, 354–363.

Hosseini SMH, Hoeft F, Kesler SR (2012). GAT: a graph-theoretical analysis toolbox for analyzing between-group differences in large-scale structural and functional brain networks. *PLoS ONE* **7**, e40709.

Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM (2012). FSL. *Neuroimage* **62**, 782–790.

Kaiser M, Hilgetag CC (2006). Nonoptimal component placement, but short processing paths, due to long-distance projections in neural systems. *PLoS Computational Biology* **2**, 0805–0815.

Kanai R, Bahrami B, Duchaine B, Janik A, Banissy MJ, Rees G (2012*a*). Brain structure links loneliness to social perception. *Current Biology* **22**, 1975–1979.

Kanai R, Bahrami B, Roylance R, Rees G (2012*b*). Online social network size is reflected in human brain structure. *Proceedings of the Royal Society B: Biological Sciences* **279**, 1327–1334.

Keltner D, Kring AM (1998). Emotion, social function, and psychopathology. *Review of General Psychology* **2**, 320–342.

Korgaonkar MS, Fornito A, Williams LM, Grieve SM (2014). Abnormal structural networks characterize major depressive disorder: a connectome analysis. *Biological Psychiatry* **76**, 567–74.

Lai CH (2014). Patterns of cortico-limbic activations during visual processing of sad faces in depression patients: a coordinate-based meta-analysis. *Journal of Neuropsychiatry and Clinical Neurosciences* **26**, 34–43.

Laird AR, Eickhoff SB, Li K, Robin DA, Glahn DC, Fox PT (2009). Investigating the functional heterogeneity of the default mode network using coordinate-based meta-analytic modeling. *Journal of Neuroscience* **29**, 14496–14505.

Leary MR (1990). Responses to social exclusion: social anxiety, jealousy, loneliness, depression, and low self-esteem. *Journal of Social and Clinical Psychology* **9**, 221–229.

Lee TMC, Leung MK, Hou WK, Tang JCY, Yin J, So KF, Lee CF, Chan CCH (2012). Distinct neural activity associated with focused-attention meditation and loving-kindness meditation. *PLoS ONE* **7**, e40054.

Lim HK, Jung WS, Aizenstein HJ (2013). Aberrant topographical organization in gray matter structural

network in late life depression: a graph theoretical analysis. *International Psychogeriatrics* **25**, 1929–1940.

Lindquist KA, Wager TD, Kober H, Bliss-Moreau E, Barrett LF (2010). The brain basis of emotion: a meta-analytic review. *Behavioral and Brain Sciences* **35**, 121–202.

Luo Y, Hawkley LC, Waite LJ, Cacioppo JT (2012). Loneliness, health, and mortality in old age: a national longitudinal study. *Social Science & Medicine* **74**, 907–914.

Masi CM, Chen H-Y, Hawkley LC, Cacioppo JT (2011). A meta-analysis of interventions to reduce loneliness. *Personality and Social Psychology Review* **15**, 219–266.

Mayberg HS (1997). Limbic-cortical dysregulation: a proposed model of depression. *The Journal of Neuropsychiatry and Clinical Neurosciences* **9**, 471–481.

Meng C, Brandl F, Tahmasian M, Shao J, Manoliu A, Scherr M, Schwerthoffer D, Bauml J, Forstl H, Zimmer C, Wohlschlager AM, Riedl V, Sorg C (2014). Aberrant topology of striatum's connectivity is associated with the number of episodes in depression. *Brain* **137**, 598–609.

Müller MJ, Dragicevic A (2003). Standardized rater training for the Hamilton Depression Rating Scale (HAMD-17) in psychiatric novices. *Journal of Affective Disorders* **77**, 65–69.

Nelson JC, Delucchi K, Schneider LS (2008). Efficacy of second generation antidepressants in late-life depression: a meta-analysis of the evidence. *American Journal of Geriatric Psychiatry* **16**, 558–567.

Norris CJ, Chen EE, Zhu DC, Small SL, Cacioppo JT (2004). The interaction of social and emotional processes in the brain. *Journal of Cognitive Neuroscience* **16**, 1818–1829.

Oldfield RC (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97–113.

Perissinotto CM, Stijacic Cenzer I, Covinsky KE (2012). Loneliness in older persons: a predictor of functional decline and death. *Archives of Internal Medicine* **172**, 1078–1083.

Powers KE, Wagner DD, Norris CJ, Heatherton TF (2013). Socially excluded individuals fail to recruit medial prefrontal cortex for negative social scenes. *Social Cognitive and Affective Neuroscience* **8**, 151–157.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences USA* **98**, 676–682.

Rissman J, Gazzaley A, D'Esposito M (2004). Measuring functional connectivity during distinct stages of a cognitive task. *Neuroimage* **23**, 752–763.

Rubinov M, Sporns O (2010). Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* **52**, 1059–69.

Russell DW (1996). UCLA Loneliness Scale (version 3): reliability, validity, and factor structure. *Journal of Personality Assessment* **66**, 20–40.

Sexton CE, MacKay CE, Ebmeier KP (2013). A systematic review and meta-analysis of magnetic resonance imaging studies in late-life depression. *American Journal of Geriatric Psychiatry* **21**, 184–195.

Sheline YI, Barch DM, Price JL, Rundle MM, Vaishnavi SN, Snyder AZ, Mintun MA, Wang S, Coalson RS, Raichle ME (2009). The default mode network and self-referential processes in depression. *Proceedings of the National Academy of Sciences USA* **106**, 1942–1947.

Steffens DC, Taylor WD, Denny KL, Bergman SR, & Wang L (2011). Structural integrity of the uncinate fasciculus and resting state functional connectivity of the ventral prefrontal cortex in late-life depression. *PLoS ONE* **6**, e22697.

Tadayonnejad R, Ajilore O (2014). Brain network dysfunction in late-life lepression: a literature review. *Journal of Geriatric Psychiatry and Neurology* **27**, 5–12.

Tian Y, Liang S, Yuan Z, Chen S, Xu P, Yao D (2014). White matter structure in loneliness. *NeuroReport* **25**, 843–847.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, & Joliot M (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* **15**, 273–289.

VanderWeele TJ, Hawkley LC, Thisted RA, Cacioppo JT (2011). A marginal structural model analysis for loneliness: implications for intervention trials and clinical practice. *Journal of Consulting and Clinical Psychology* **79**, 225–235.

Yeh F-C, Verstynen TD, Wang Y, Fernández-Miranda JC, Tseng W-YI (2013). Deterministic diffusion fiber tracking improved by quantitative anisotropy. *PLoS ONE* **8**, e80713.

Yuan Y, Zhang Z, Bai F, Yu H, Shi Y, Qian Y, Liu W, You J, Zhang X, Liu Z (2008). Abnormal neural activity in the patients with remitted geriatric depression: a resting-state functional magnetic resonance imaging study. *Journal of Affective Disorders* **111**, 145–152.

Zalesky A, Fornito A, & Bullmore ET (2010). Network-based statistic: identifying differences in brain networks. *Neuroimage* **53**, 1197–1207.